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Prof. Dr hab. Anna Goździcka-Józefiak

Senior professor

Department of Molecular Virology

Adam Mickiewicz University of Poznan

Review

of Mamata Sumant Savanagouder MSc's PhD thesis titled - "Furthering the understanding of the mechanism and function of the HCMV IE1 chromatin association"

Mamata Sumant Savanagouder MSc wrote this PhD thesis under the supervision of Magdalena Weidner-Glunde, an assistant professor in the Department of Reproductive Immunology & Pathology at the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn.

The aim of the thesis was to study the mechanism of the association of Human Cytomegalovirus (HCMV) IE1 protein with the cellular chromatin.

HCMV belongs to the Herpesviridae group of viruses and has a large, double-stranded DNA genome with approximately 230kb that encodes for more than 170 proteins, as well as numerous RNA. Generally, infections with the human cytomegalovirus are linked to a form of infectious mononucleosis, congenital infection and birth defects. This virus is a major factor in the mortality of organ transplant patients. HCMV can also induce a transformation of human embryonic lung fibroblast or human mammary epithelial cells, and can act as an oncomodulatory factor. HCMV can cause lytic as well as latent infections. Latency is a key feature of HCMV pathogenesis. The virus genome is maintained as an extrachromosomal, covalently closed circular episome within latently infected nuclei. HCMV latency exhibits expressions of several immediate early

(IE) and early genes, but the role of its products in the maintenance of latency is unknown. The IE1 of HCMV is required for viral early gene expression and has been suggested that it may serve as a tethering protein for HCMV episomes to host chromosomes.

The IE1 of HCMV also binds to cellular restriction factor promyelocytic leukemia protein (PML) and antagonizes its repressive activity on viral gene expression. The IE1 protein is a nuclear phosphoprotein of 491 amino acids which consists of the N terminal domain (NTD), core domain (with the residues corresponding to nuclear localization signals), acidic domain (AD), and chromatin tethering domain (CTD). There are different isoforms IE1 -IE1 72kDa, 1E1x4 60 kDa, IE19 kDa,IE17,5 kDa and IE 9kDa. It has also been proposed that a short IE1 isoform referred to IE1 exon 4 (IE1x4 60kDa) may also serve as a tethering protein for HCMV episomes with the cellular chromatin, and it may be a functional homolog of EBV EBNA1 and KSHV LANA of gammaherpesviruses maintenance proteins. The tethering of a viral genome to host chromosomes has been recognized in a variety of DNA and RNA viruses, however the mechanism in cells latently infected with HCMV remains unknown. For this reason, the purpose of the research undertaken by Mamata Sumant Savanagouder MSc is justified and important.

One of the first aims of the PhD student was to analyze the localization pattern of IE1 and IE1x4 in the cells in the presence of HCMV TR, which is required for viral maintenance and replication.

Mamata Sumant Savangouder started these studies by amplifying the IE1 gene, its variants, and HCMV terminal repeat regions (TR1 and TR2) using PCR methods. The respective PCR products were cloned in appropriate plasmids for cloning and the expression of the tagged protein (EGFP-C1, pcDNA) in a variety of mammalian cells.

Mamata Sumant Savangouder indicated the expression of both IE1 and IE1x4 proteins in transfected T98G (derived from a human glioblastoma are a model for HCMV latency) and HeLa cells used specific antibodies. The stained proteins IE1x4 and IE1 were localized in the nucleus during interphase in T98G and HeLa cells and to the chromosomes during mitosis. The localization pattern of IE1x4 was not changed in the study cells in the presence of the HCMV TR region during interphase (HeLa cells, MRC-5 (derived from embryonic lung tissue)), T98G cells), and during mitosis in HeLa and T98G cells. These results do not support the suggestion that IE1x4 can act as the protein tethering HCMV genome through the TR sequence in study cell types in a manner analogous to LANA of KSHV. In the course of further study Mamata Sumant Savangouder analyzed the expression of HCMV proteins in T98G and KASUM-3 cells (Lymphoblast cells derived from acute myeloblastic) after infection of HCMV. Analysis of the viral gene transcripts from 7dpi to 35dpi, by qRT-PCR, and proteins by

immunofluorescence staining and immunoblotting indicated expression in the immediate early gene IE1, IE2, UL44 and gB but not 1Ex4 protein. IE1, but not IE1x4 was also expressed in latent KASUM-3 cells. The PhD student also performed an infectivity assay which did not indicate the viral particle production by infected T98G cells past 7dpi. In the following experiments, Mammata Sumant Savangouder indicated the localization pattern of IE1 in the form of chromosomal-associated spots (CAS) on a few chromosomes. Whereas KSHV LANA, a known gene maintenance protein, forms multiple spots on both arms of all chromosomes.

The PhD student also observed IE1CAS in U87 MG cells (derived from embryonic lung tissue), and TH81 cells on 8-10% of chromosomes but not in HeLa cells, human placental fibroblast (HPF), neural stem cells, and colon cancer (HT29). It suggests that IE1CAS is formed, above all, in cell tumor origin that can support HCMV latency. According to the doctoral student, IE1CAS may be at the sites of HCMV genome tethering in T98G cells during the latent phase of infection. Mamata Sumant Savangouder indicated that HCMV IE1 spots are localized in the pericentromeric region of the chromosomes and also colocalized with PML.

The results of this study suggest that for association of IE1 protein with mitotic chromosomes can be responsible for not only IE1 chromatin tethering domain (CTD), and specifically the nucleosome motif (NBM), but a novel domain can exist as the second chromatin binding region independent of CDT. Therefore, Mammata Sumant Savangouder generated two IE1 mutants: one with lacking critical residues in the NBM binding motif, and IE1 without an NBM motif. Both mutated IE1s in transfected T98G cells were localized to the nucleus in same manner as IE1 WT. These results confirmed the suggestion that a novel domain independent of CTD exists, which is involved in CAS formation on chromosomes. In the course of further study the PhD student tried to identify this domain. For this purpose Mammata Sumant Savangouder prepared a series of c-myc and EGFP tagged IE1 constructs including: individual domain_ core domain (CD), acidic domain (AD), and the chromatin tethering domain (CTD), a fusion of AD and CTD and IE1 construct lacking exon 2 and exon 3 regions and construct lacking the N-terminal domain. The expression of these constructs was analyzed in transfected T98G cells by immunoblotting. Among study constructs, only the CD localized in the nucleus appeared as spots on mitotic chromosomes. Mammata Sumant Savangouder, using specific mutants IE1CD, also identified the core domains that are not involved in IE1CAS, and indicated that the localization pattern of IE1 HCMV in transfected T98G cells is dependent on IE1 protein expression and may change upon latency. I consider it an interesting set of results in which the doctoral student is able to demonstrate that localization of IE1CAS is maintained during cell division.

Summarizing the research results, I believe that the research objectives set at the stage of the dissertation have been fully achieved.

The reviewed work contains a range of new data regarding HCMV latency.

Mammata Summant Savangouder indicated:

-for the first time that IE1x4 most probably does not mediate genome tethering in a manner similar to that of KSHV LANA,

- HCMV TR is not the maintenance element of this viral genome,

- in IE1 binding to histones participates in the nucleosome binding motif (NBM) and the core domain as the second chromatin binding region of IE1

The PhD thesis of Mammata Sumant Savangouder was very well planned and systematically implemented. The results obtained in this dissertation are presented on 45 pages, and are documented in photographs (20), figures (23) , tables (10) and competently discussed related to the literature on the subject (272 items).

The implementation of presented studies required the use of a lot of modern research techniques of molecular biology, virology, immunology, and the use of the confocal microscope. The range of these methods used is very wide. All the methods used in the work are described very carefully, in particular the materials and methods used. All techniques were adequate to the research goals.

The PhD thesis arrangement is typical for doctoral thesis. The experimental part of work is preceded by a theoretical introduction presenting the latest information on the nature of HCMV, and its pathogenicity.

In my opinion the dissertation furthers the understanding of the mechanism and function of HCMV IE1 chromatin association, and contributes to a valuable scientific achievement of Mammata Sumant Savangouder through the original contribution to our knowledge regarding to the mechanism and function of IE1 of HCMV in latency of virus. This work deserves appropriate recognition.

Rozprawa doktorska Pani mgr Mamaty Savangouder pt. „ **Furthering the understanding of mechanism and function of HCMV IE1 chromatin association"**

spełnia warunki określone w art.187 ust.1-4 Ustawy Prawo o szkolnictwie wyższym i nauce (Dz.U.2018 poz.1668 z późn.zm)

Wnoszę zatem do Wysokiej Rady Instytutu Immunologii i Terapii Doświadczalnej im Ludwika Hirszfelda Polskiej Akademii Nauk Centrum Doskonałości : Immune wniosku o dopuszczenie mgr Mamaty Savangouder do dalszych etapów przewodu doktorskiego. Ze względu na wysoki walor poznawczy praca zasługuje na wyróżnienie stosowną nagrodą.

Prof.dr hab. Anna Goździcka-Józefiak