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ABSTRACT BOOK

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Citrullination and Herpes Simplex Virus type 1 infection: implications for neurodegenerative disorders

Selina Pasquero (1), Gloria Griffante (2), Francesca Gugliesi (1), Matteo Biolatti (1), Camilla Albano (1), Sergio Fernando Castillo Pacheco (1), Marco De Andrea (3), Santo Landolfo (1), Valentina Dell'Oste (1)

(1) Department of Public Health and Pediatric Sciences, University of Turin, Turin, TO, Italy
(2) Department of Translational Medicine, University of Eastern Piedmont, Novara, NO, Italy
(3) Center for Translational Research on Autoimmune and Allergic Disease-CAAD, Turin, TO, Italy

Herpes simplex virus type 1 (HSV-1) is a neurotropic virus that infects most humans, attaining 90% prevalence by the sixth decade of life. The virus remains latent in neuronal cell bodies and reactivates due to stress, illness, and other unknown factors throughout an individual's life. The disease appears as cold sores, typically seen on the lips or face, with primary infection usually during childhood. In some cases, individuals can develop adverse reactions such as herpes simplex encephalitis (HSE) and recent evidence suggests the involvement of HSV-1 in the etiology of Alzheimer's disease (AD). Sporadic AD is a complex multifactorial neurodegenerative disease with evidence indicating coexisting multi-pathogen and inflammatory etiologies. Citrullination is a post-translational modification (PTM) catalyzed by peptidyl arginine deiminases (PAD) that convert peptidylarginine into peptidylcitrulline, whose dysregulation has been associated with Alzheimer's disease and others neurodegenerative disorders. Against this background, the goal of this project is to characterize the citrullination during infection with HSV-1 in different in vitro models. We demonstrate that HSV-1 triggers PADs expression in Human Foreskin Fibroblasts (HFF), African green monkey kidney cells (VERO) and Human Neuroblastoma Cell Line (SHSY-5) cell lines both at mRNA and protein levels. Furthermore, the overall citrullination profile obtained with the citrulline-specific rhodamine phenylglyoxal (RhPG)-based probe changes consistently at different time points during infection in all tested cell lines. Finally, HSV-1 replication rate is strongly impaired in the presence of Cl-amidine and BB-Cl-amidine, two specific pan-PAD inhibitors, indicating that citrullination is required for HSV-1 replication. These findings could shed light on the role of HSV-1 in the pathogenesis of AD, providing new molecular mechanisms that could be exploited for advanced medical interventions.

Contact: Selina Pasquero, selina.pasquero@unito.it

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Cellular State Landscape and Heterogeneity in HSV-1 Gene Expression

Maija Pietilä (1), Cornel Fraefel (1), Lucas Pelkmans (2)

(1) Institute of Virology, University of Zurich, Zurich, Switzerland
(2) Department of Molecular Life Sciences, University of Zurich, Zurich, Switzerland

Single-cell features can predict whether a cell will be infected or not. However, there is significant cell-to-cell variability between infected cells resulting in diverse cell fates and infection outcomes. In this study, our aim was to determine which changes are induced in the cellular state landscape during Herpes Simplex Virus 1 (HSV-1) infection and which cellular features correlate with low versus high virus gene expression. HSV-1 transcription was studied using single-molecule mRNA fluorescence in situ hybridization (smFISH). In order to simultaneously follow multiple cellular state markers within the same single cells, smFISH was followed by iterative indirect immunofluorescence imaging (4i). We focused on antiviral, signaling and transcription markers as well as on subcellular compartments. HSV-1 gene expression was also followed at the protein level using 4i. Both viral transcripts and viral and cellular proteins were analysed from thousands of single cells using high-throughput automated imaging. Computer vision and image processing were used to segment cellular and viral structures and to extract single-cell features. Multiplexed time-course data acquired by this large-scale quantitative image analysis allowed us to determine infection progression in a heterogenous cellular environment. HSV-1 gene expression varied along the cellular state landscape, and we also observed a plethora of cellular changes induced by the virus. Using trajectory inference methods and multiplexed data we were able to model infection progression in the changing cellular landscape.

Contact: Maija Pietilä, maija.pietilae@uzh.ch