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Գ.00.03 – «Մոլեկուլային և բջջային կենսաբանություն» մասնագիտությամբ
կենսաբանական գիտությունների թեկնածուի գիտական աստիճանի
հայցման ատենախոսության

ՍԵՂՄԱԳԻՐ

ԵՐԵՎԱՆ – 2017

NATIONAL ACADEMY OF SCIENCES OF REPUBLIC OF ARMENIA
INSTITUTE OF MOLECULAR BIOLOGY

IZMAILYAN ROZA ARTASHES

INVESTIGATION OF VACCINIA VIRUS ENTRY PATHWAYS

SYNOPSIS

Submitted for the degree of candidate of biological sciences in the specialization
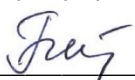03.00.03 - "Molecular and Cellular Biology"

YEREVAN 2017

Ատենախոսության թեման հաստատվել է ՀՀ ԳԱԱ Մոլեկուլային կենսաբանության ինստիտուտի գիտական խորհրդում:

Գիտական ղեկավար՝   կենս. գիտ. թեկնածու, պրոֆ. Վ. Չանգ

Պաշտոնական ընդդիմախոսներ՝   կենս. գիտ. դոկտոր, պրոֆ.,
                                    ՌԳԱ թղթ.-անդամ Ե.Ա. Կրասավին
                                    կենս. գիտ. դոկտոր, պրոֆ. Տ.Կ. Դավթյան

Առաջատար կազմակերպություն՝   ՀՀ ԳԱԱ "Հայկենսատեխնոլոգիա"
                                    գիտաարտադրական կենտրոն

Ատենախոսության պաշտպանությունը տեղի կունենա 2017թ. հուլիսի 3-ին, ժամը 15^{00}-ին ՀՀ ԳԱԱ Մոլեկուլային կենսաբանության ինստիտուտում, Կենսաքիմիայի 042 մասնագիտական խորհրդում (ՀՀ, 0014, ք.Երևան, Հասրաթյան 7):

Ատենախոսությանը կարելի է ծանոթանալ ՀՀ ԳԱԱ մոլեկուլային կենսաբանության ինստիտուտի գրադարանում և http://molbio.sci.am կայքում:

Սեղմագիրն առաքվել է 2017թ. հունիսի 2-ին:

042 մասնագիտական խորհրդի գիտական քարտուղար
կենս. գիտ. թեկնածու   Գ.Մ. Մկրտչյան
_____

The theme of the thesis was approved at the scientific council of the Institute of Molecular Biology, NAS RA.

Scientific supervisor: PhD, Professor W. Chang
Official opponents: D.Sc., Professor, RAS corr. member E.A. Krasavin
                                    D.Sc., Professor T. K. Davtyan
Leading organization: "Armbiotechnology" Scientific and
                                    Production Center of NAS RA

Thesis defense will take place on July 3 rd 2017, at 15^{00} PM, at the meeting of the specialized council 042 on biochemistry at the Institute of Molecular Biology NAS RA (7 Hasratyan str., 0014, Yerevan, RA).
The thesis will be available in the library of the Institute of Molecular Biology
NAS RA as well as at the http://molbio.sci.am website.
Synopsis was submitted on June 2nd 2017.
Scientific secretary of the specialized council 042,
PhD   G.M. Mkrtchyan

Relevance of the work. Vaccinia virus is the prototype of the orthopoxvirus genus in the family of Poxviridae. Poxviruses differ from other animal viruses in their large size and complexity. The well-known member of this family is Variola virus, the causative agent of smallpox disease in humans that took millions of human lives over the millenniums, until was eradicated by global vaccination. Although in 1980 the World Health Organization declared that smallpox is eradicated from natural environment it is still an emerging subject to be investigated because the virus stocks of the original smallpox are still kept by various laboratories in Russian Federation and the United States and remains as a bioterrorism agent. Moreover, it is known that the other poxviruses also can be transmitted from animals to human and cause fatal diseases in human population. An example is monkeypox virus, which can transmit to people through a variety of wild animals and produce a smallpox-like disease suggesting that monkeypox might replace smallpox as a serious epidemic threat [Breman $ Henderson, 1998]. Therefore, nowadays, the development of new antiviral agents and the enlargement of the information about virus entry and replication are of most importance.

Vaccinia virus is a large ~200kb, double-stranded, enveloped, DNA virus that encodes more than 200 open reading frames (ORF). It has a broad host range of infectivity both *in vitro* and *in vivo*, infects many cell lines, humans and animals. Virion morphogenesis occurs exclusively in the cytoplasm of the host cells and produces several forms of infectious particles: mature virus (MV), wrapped virus (WV) and extracellular virus (EV). Previous work in our laboratory showed that cell-bound Vaccinia MV particles were clustered at the plasma membrane lipid rafts prior to virus entry and that the interruption of lipid raft integrity with methyl-β-cyclodextran significantly reduces Vaccinia MV entry into HeLa cells [Chung et al., 2005]. Since lipid rafts on the plasma membrane are known to act as platforms for receptor clustering, endocytosis and signal transduction for many viruses [Rauch $ Fackler, 2007], it is possible that cellular proteins within plasma membrane lipid rafts may mediate Vaccinia MV cell entry.

Purpose and objectives of the study. The main goal of this study was to reveal the specific molecular and cellular mechanisms, pathways, cellular receptors and proteins involved in Vaccinia virus attachment and penetration processes.

To achieve this goal, the following tasks were accomplished:

- Identification of the cellular receptors and proteins within lipid raft microdomains involved in the Vaccinia MV entry process by the investigation of their association with Vaccinia MV on the surface of infected HeLa cells;
- Investigation of the role of these receptors and proteins in the Vaccinia virus life cycle and Vaccinia MV entry process into HeLa and mouse cells;
- Investigation of the pathways and mechanisms through which these receptors and proteins mediate Vaccinia MV cell penetration and entry process.
- Identification and investigation of the functions of two Vaccinia MV proteins that previously were unknown.

Scientific and practical significance of the [3] **study:** It was shown that the raft-associated

protein, integrin β1 (ITGβ1), associates with Vaccinia MV on HeLa cells. Moreover, Vaccinia virus entry is reduced in ITGβ1 siRNA KD HeLa cells and mouse cells lacking ITGβ1 expression. More importantly, Vaccinia MV triggers the ITGβ1-dependent activation of intracellular phosphatidylinositol 3-kinase (PI3K) signaling leading to virus endocytosis into HeLa cells. Furthermore, the outside-in activation of ITGβ1 function facilitates Vaccinia virus entry into HeLa cells, since the disruption of focal adhesions also reduced Vaccinia MV entry. The results obtained also showed that ITGβ1 is essential for the adhesion, spreading and migration of HeLa cells.

In addition to integrin β1, a new component of intracellular Vaccinia MV, *WR53.5/F14.5* protein was identified, was shown to be conserved in other poxvirus family members and the ORF of which encodes a late viral envelope protein in MV particles. Recombinant vi53.5L virus infection of BSC40 cells showed that even though *WR53.5* protein does not participate in plaque formation and MV/EEV production in infected cells it regulates the cell morphology and Ca^{2+}-independent cell adhesion in vitro and virus virulence in vivo.

Another new component of Vaccinia MV, is an envelope *WR-G3* protein, was also identified, which was shown to be important for Vaccinia virus cell entry. Particularly, the conserved Vaccinia *G3L* gene encodes a late viral envelope protein associated with MV particles and MV membrane component. Using recombinant virus we reviled that its expression is tightly regulated at the late phase by IPTG. Although MV particles devoid of G3 have normal morphology and major protein content we showed that G3 is required for plaque formation and MV production in cell culture, is essential for MV penetration into cells and for cell-cell fusion induced by low-pH treatment but it is not required for virion morphogenesis of MV and EV and for CEV formation.

Thus, our study for the first time identified that cellular receptor ITGβ1 mediates Vaccinia virus endocytosis, through PI3K activation. A new Vaccinia MV envelope protein, *WR-53.5,* that mediates cell adhesion and is important for virus virulence *in vivo*, as well as Vaccinia G3 protein as an essential component of entry fusion complex.

The revelation of the Vaccinia virus cellular receptors and proteins not only sufficiently enlarges and complements the existing knowledge on the molecular and cellular mechanisms of the Vaccinia virus cell penetration and entry processes, but also helps to develop new antiviral agents against Vaccinia virus, to develop new strategies for the prevention of the infection and for treatment of infected individuals.

The approbation of the work: The main results of this dissertation have been extensively discussed with the experts in the field and presented in the seminars organized by the Dr. Wen Chang's Laboratory, Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan R.O.C. (Taipei, Taiwan, 2006-2011), and by the Laboratory of Cell Biology and Virology (head, D.Sc. Zaven Karalyan) of the Institute of Molecular Biology NAS RA, at the meetings of the scientific council of the Armenian institution (Yerevan, RA, 2012-2016), as well as at 12 international scientific conferences, symposiums and meetings, such as: International FASEB Summer Research Conference, Poxviruses (California, USA, 2006), International Meeting of the Molecular Biology in the XXI Century: Interface, Integration and Perspectives (Taipei, Taiwan, R.O.C., 2006), XVII International Poxvirus and Iridovirus[4]Conference (Grainau-Bavaria, Germany, 2008), Institute of Molecular Biology Retreat Meeting (Kaoshong, Taiwan, R.O.C., 2008), International

Conference of American Society for Virology: XVIII Annual Meeting (BC, Canada, 2009), Institute of Molecular Biology Retreat Meeting (Taoyuan, Taiwan, R.O.C., 2009), XII SCBA International Symposium Science for a Healthier and Better Life (Taipei,Taiwan, R.O.C. 2009), XVIII International Poxvirus, Asfivirus and Iridovirus Symposium (Sedona, USA, 2010), Institute of Molecular Biology Retreat Meeting (Taoyuan, Taiwan, R.O.C., 2011), International Meeting Gordon Research Conference (GRC), Viruses and Cells (Lucca-Barga, Italy, 2011), International Conference of American Society for Virology: 30th Annual Meeting (Minneapolis, USA, 2011), XIX International Poxvirus and Iridovirus Conference (Salamanca, Spain, 2012).

Publications: The main results of this study are involved in 10 scientific publications, including 4 articles in high-impact factor peer-reviewed journals, 1 article in Armenian journal, and 5 reports in abstract books of international conferences.

The volume and structure of the thesis: The dissertation comprises of 114 pages, includes 1 table and 30 figures, and consists of the following sections: List of Abbreviations and Symbols, Introduction, Literature Review, Materials and Methods, Results and Discussion, Conclusions, Inferences and References. The list of references includes 160 cited literature sources.

MATERIALS AND METHODS

This work was done at the Dr. Wen Chang's laboratory, Institute of Molecular Biology, Academia Sinica, Taipei Taiwan R.O.C. Organization and writing of the dissertation was accomplished at the Laboratory of Cell Biology and Virology (head, D.Sc. Zaven Karalyan) of the Institute of Molecular Biology, NAS RA.

Study objects: Five cell lines, GD25, GD25β1A, HeLa, BSC40 and BSC1 for in vitro and BALB/c mice for in vivo studies were used for infection, wild type Western Reserve strain of Vaccinia virus (WR-VV) as well as recombinant viruses generated for this study by Izmailyan and co authors.

GD25 cell line: It was derived from ITGβ1 knockout (KO) embryonic stem cells [Fassler et al., 1995]. **The stably transformed cell line GD25β1A** resulted from GD25 electroporation with wild type human ITGβ1 cDNA. HeLa, BSC40, BSC1, GD25 and GD25β1A cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 2% penicillin/streptomycin (Gibco) in a 5% CO_2 incubator at 37°C. **The WR-VV strain** was purified through sucrose gradient purification. **Recombinant plasmids** were cloned to generate of vi53.5, viG3L IPTG inducible recombinant viruses for *in vitro* and WR Δ53.5 virus for *in vivo* experiments.

ITGβ1-mediated Vaccinia virus entry: The identification of cellular proteins within lipid raft microdomains of HeLa cells for quantitative proteomic analyses was performed by stable isotope labeling, [Schroeder et al., 2012]. **The association of ITGβ1 with Vaccinia MV on HeLa cells** was studied by co patching experiment using Anti- ITGβ1 and anti-vaccinia MV antibodies as described previously by {Huang et al., 2008]. Immunoblots, luciferase and FACS analyses as well as Vaccinia MV binding assays and virus core uncoating assays by Immunofluorescence analyses

5

[Vanderplasschen et al., 1997] were conducted **to test the role of ITGβ1 in Vaccinia MV entry into HeLa cells and mouse cell line GD25β1A**, Small interfering RNA (si RNA) approach were conducted in HeLa cells, control siRNA (si-cont), siRNA targeting ITGβ1 (si-ITGβ1) or cyclophilin B (si-CypB). Immunoblot analyses with anti-phospho-Akt were performed to study the **activation of PI3K/Akt signaling in GD25β1A cells**. **To turn on the ITGβ1 -dependent signaling pathway in HeLa cells** outside-in activation experiments were performed using extracellular matrix proteins fibronectin (FN) and laminin (LN) as described earlier by [Schiller et al., 2011}. To address if ITGβ1 is essential to mediating HeLa cell activation on extracellular matrix proteins, adhesion and spreading experiments from HeLa ITGβ1 KD cells were performed using HeLa si-control and si-ITGβ1 KD cells [Wennerberg et al., 2000; Izmailyan et al., 2012].

The role of *WR53.5/F14.5* protein for Vaccinia virus entry: **Expression of the wild-type WR53.5 protein was monitored** in BSC40 cells infected with Vaccinia virus WR-2 by previously described method [Izmailyan et al., 2006]. **The presence of WR53.5 protein in the membrane fraction of MV** was analyzed via the extraction of purified wild type WR-2 MV. **The role of WR53.5L in Vaccinia virus life cycle** was examined in BSC40 cells infected with recombinant vi53.5L in the presence or absence of IPTG. *WR53.5* protein mediated cell morphology and adhesion of virus-infected BSC40 cells were studied using the methods described elsewhere [Izmailyan et al., 2008]. Immunofluorescent assay was used to detect cell surface expressed *WR53.5* protein. Extracellular calcium depletion assay was performed as described previously [Beard et al., 2006].

Anti-G3L antibody was used to study **the expression of *G3L* during Vaccinia virus infection**. Extraction of purified MVs was conducted **to determine whether *WR53.5 or G3L* is present in the membrane fraction of MV** [Chiu $ Chang, 2002]. **The role of *G3L* during the Vaccinia virus life cycle in cell culture** was explored using a recombinant Vaccinia virus, viG3L, that expressed G3L conditionally regulated by IPTG [izmailyan et al., 2006].

Imaging experiments: All fluorescence images were collected with an LSM510 Meta Confocal Laser Scanning Microscope (Carl Zeiss Germany) using a 63x objective lens.

Statistical analyses were performed using Student's t-test in Prism software (GraphPad). The *P*-value is shown **, $P < 0.001$., ***, $P < 0.0001$.

RESULTS AND DISSCUSIONS

1. Integrin β1 mediates Vaccinia virus entry through activation of PI3K/akt signaling

Association of ITGβ1 with Vaccinia MV on HeLa cells: 570 cellular proteins within lipid raft microdomains were previously identified in our laboratory by [Schroeder at al., 2012], from which proteins with altered levels after Vaccinia virus infection constitute about 3%. Here, the remaining 97% of "constitutive" raft-associated proteins were analyzed. The biological network analyses (Figure 1) revealed the presence of ITGβ1 and its associated proteins (CD9, CD47, CD59, CD98,

talin, ezrin, Fyn/yes/lyn) suggesting that ITGβ1-mediated signaling may participate in Vaccinia MV entry. Moreover, it was shown that Vaccinia MV co-patched with cell surface ITGβ1 in HeLa cells (Figure 2), at 1 hr binding stage, suggesting a role of ITGβ1 in Vaccinia MV attachment.
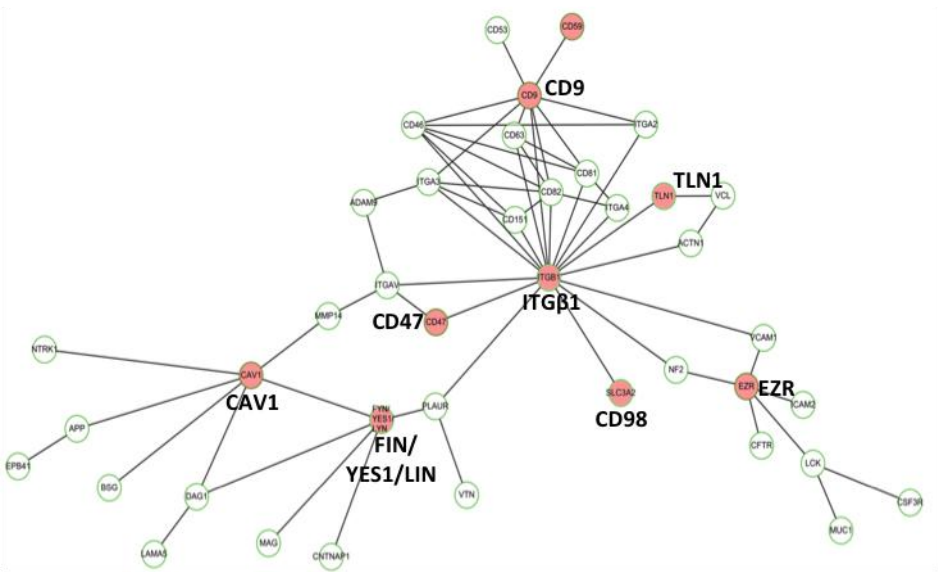

Figure 1. Hierarchical ITGβ1 network. ITGβ1 and its associated proteins (CD9, CD47, CD59, CD98, talin, ezrin, Fyn/yes/lyn). (red marks). Constructed by ARIADNE Pathway Studio 7.0 software
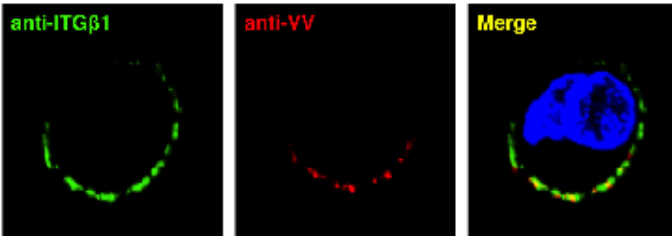

Figure 2. Vaccinia MV co localizes with ITGβ1 at the surface of HeLa cells.

Vaccinia virus entry in ITGβ1 knockdown HeLa cells. The transfection of si-cont, si-ITGβ1 or si-CypB into HeLa cells showed that si-ITGβ1 and si-CypB constructs specifically knock down (KD) the total amounts of ITGβ1 and7cyclophilin B proteins, respectively (Figure 3A). In addition, ITGβ1 expression on cell surface was reduced in si-ITGβ1 KD but not in si-cont

and si-CypB KD cells (Figure 3B). Si-ITGβ1 also affected ITGβ1-mediated cell adhesion leading to alterations in cell morphology and disorganization of focal adhesions (Figure 3C) that confirms the specificity of si-ITGβ1. Moreover, Vaccinia virus infection of si-ITGβ1 KD cells is reduced to 40% (at 2h p.i.) and 38% (at 4h p.i.) of the infection rates seen in the si-cont and si-CypB KD cells (Figure 3D) suggesting that the reduction is not due to a delayed kinetics. Finally, it was obtained that MV attachment is reduced to 52% in si-ITGβ1 KD cells to 32% (Figure 3F) indicating ITGβ1 importance for Vaccinia virus entry at both attachment and penetration steps.
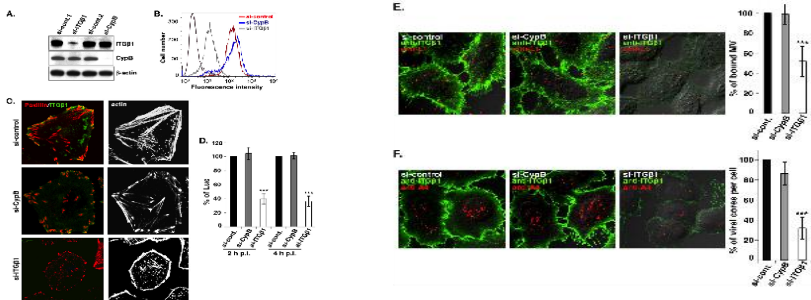

Figure 3. Vaccinia virus infection in si-ITGβ1 KD HeLa cells

   The investigation of Vaccinia virus entry in mouse cells lacking ITGβ1 expression: In the control we showed that ITGβ1 is expressed only on the surface GD25β1A cells but not on the surface of GD25 cells (ITGβ1 KD) (Figure 4A). Consequently, when the cells were exposed with the similar amount of virus, abundant MV particles bound to GD25β1A cells and colocalized with surface ITGβ1 were concentrated at cellular protrusions, whereas fewer MV were bound to GD25 cells (Figure 4B). Vaccinia MV entry into GD25 cells also was less efficient than into GD25β1A cells (Figure 4C). Further, Vaccinia MV produces fewer plaques (30%) in GD25 cells as compire to GD25β1A cells (Figure 4D). Plaques formed in GD25 cells also are smaller than in GD25β1A cells. Thus, ITGβ1 is important in Vaccinia virus spreading among cells and mediates Vaccinia MV infections.
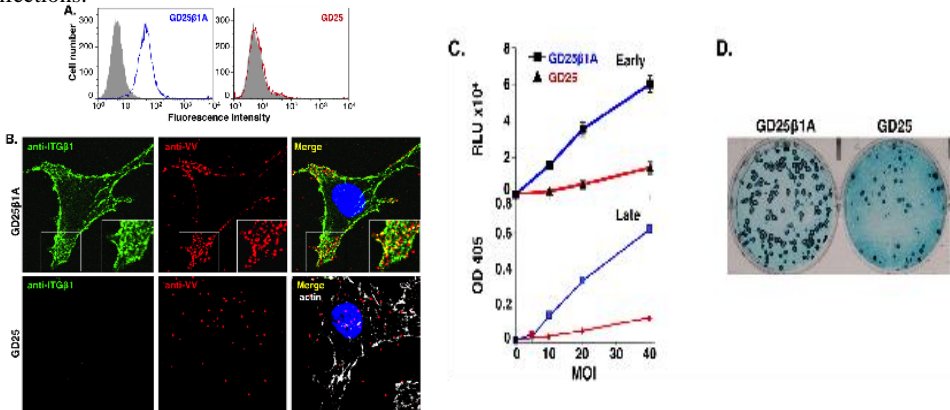

Figure 4. Vaccinia virus infection in GD25β1A and GD25 cells.

ITGβ1-mediated PI3K/Akt activation followed by virus endocytosis in HeLa cells: We test if Vaccinia MV particle activates PI3K/Akt signaling in GD25β1A cells and whether such kinase activation is critical for Vaccinia MV entry. Thus, Vaccinia MV stimulates the robust phosphorylation of Akt in GD25β1A cells 5 min after the addition of virus, compared to that in medium alone (Figure 5A). The pretreatment of GD25β1A cells with the PI3K inhibitor LY294002 completely abolished MV-induced Akt phosphorylation, showing that Vaccinia MV infection triggers the activation of Akt through PI3K. The pretreatment of GD25β1A and HeLa cells with inhibitors blocking PI3K (LY294002) and Akt (Akt IV) activities showed that PI3K and Akt inhibitors reduce Vaccinia MV infections in both GD25β1A and HeLa cells in a dose-dependent manner (Figure 5B), suggesting the importance of PI3K/Akt signaling for Vaccinia MV entry. The infection of si-cont and si-ITGβ1 KD HeLa cells with Vaccinia MV induced the phosphorylation of Akt in si-cont HeLa cells although with slower kinetics than in GD25β1A cells (Figure 5C).

In contrast, the phosphorylation of Akt was significantly reduced in si-ITGβ1 KD HeLa cells. Finally, a dosage-dependent reduction of plaque numbers was observed in LY294002 and not the DMSO control treated cells (Figure 5D). These results demonstrate that Vaccinia MV-induced PI3K/Akt activation is mediated through ITGβ1 and is required for virus entry and plaque formation.
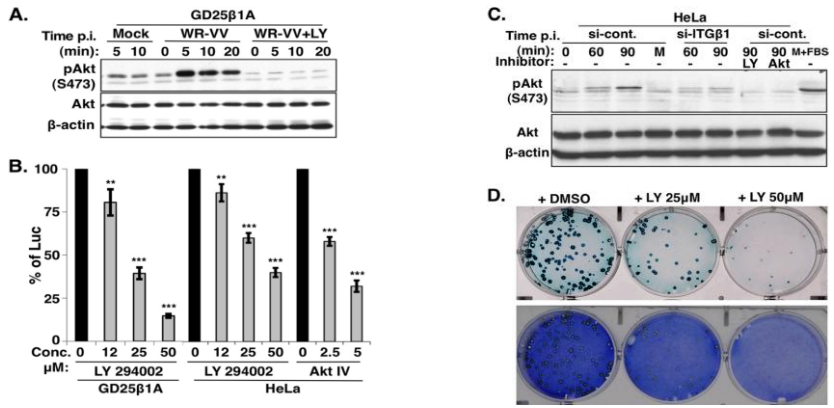

Figure 5. Integrin β1-mediated WR-Vaccinia virus activated PI3K/Akt phosphorylation (A, C). Pharmacological inhibitors of PI3K reduce virus entry (B,D)

The role of PI3K/Akt activation in Vaccinia virus endocytosis: Vaccinia virus MV enters the cell through fluid-phase endocytosis/macropinocytosis or plasma membrane fusion [Huang et al., 2008; Merser et al., 2008; Chang et al., 2010] and the references therein. The results obtained here demonstrated that control HeLa cells treated with DMSO are infected, and abundant viral cores are detected in the cytoplasm (Figure 6A, a). Moreover, BFLA pretreatment significantly reduces viral core number in cells, confirming virus entry9through a low-pH dependent endocytic process (Figure 6A, b). Pretreatment of HeLa cells with LY 294002 also reduced viral cores in cells,

suggesting the importance of PI3K/Akt for Vaccinia virus uncoating (Figure 6A, c). Finally, exposure of these cells to a low pH buffer converted MV entry through plasma membrane fusion resistant to inhibition by BFLA (Figure 6A, d) and LY 294002 (Figure 6A, e).
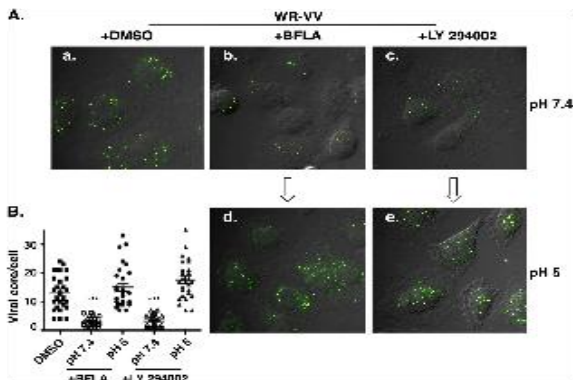

Figure 6. PI3K/Akt pharmacological inhibitor blocks Vaccinina MV endocytosis on HeLa cells.

Outside-in activation of *ITGβ1* in Vaccinia virus entry: The fact that the integrin network is essential for cell viability, migration, and growth [Legate et al., 2006] suggests, that Vaccinia MV exploits the integrin/PI3K/Akt signaling pathway to modulate cellular environments preferable for viral entry and growth. The results obtained revealed the specific activation of integrin/PI3K/Akt through cell-matrix interactions (Figure 7A). As expected, immunofluorescence staining with anti-paxillin (focal adhesion marker) antibody revealed the formation of focal adhesions in cells plated onto FN and LN but not onto PLL (Figure 7B). When these cells were infected with Vaccinia MV, the early luciferase activity level was higher in HeLa cells plated onto FN and LN than in those plated onto PLL (Figure 7C). These results demonstrate that the outside-in activation of *ITGβ1*-mediated PI3K signaling is important for Vaccinia MV entry into HeLa cells. Integrin adhesome formation induced by outside-in activation was shown previously to be disrupted upon blebbistatin treatment [Schiller et al., 2011], Interesingly, the formation of focal adhesions was completely dispersed in blebbistatin treated cells (Figure 7D), and dosage dependent redaction of virus entry was observed in this cells (Figure 7E).

Figure 7. Outside-in integrin activation enhances vaccinia virus entry.
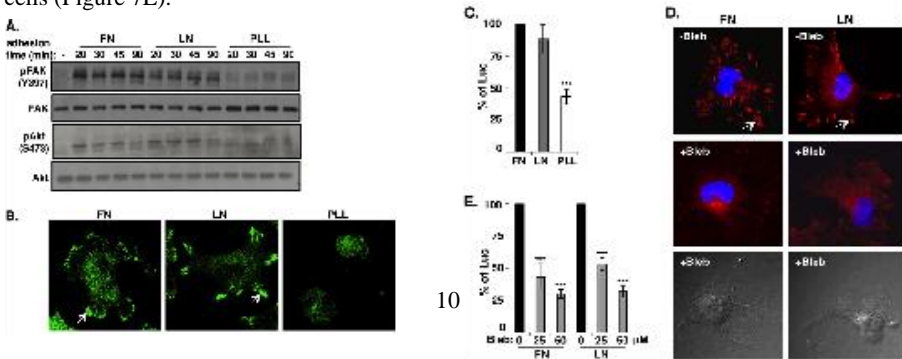
2. The role of ITGβ1 in adhesion, spreading and migration of HeLa cells:

To address if ITGβ1 is essential to mediating HeLa cell activation on extracellular matrix proteins we performed adhesion and spreading experiments from HeLa ITGβ1 KD cells. According to the results, although both si-cont and si-ITGβ1 KD cells adhere and spread to FN (Figure 8A-ab) however, si-ITGβ1 KD cells have smaller shape and disorganized cytoskeleton. In contrast, si-ITGβ1 KD cells attached to laminin poorly and those attached cells did not spread at all (Figure 8A-cd).
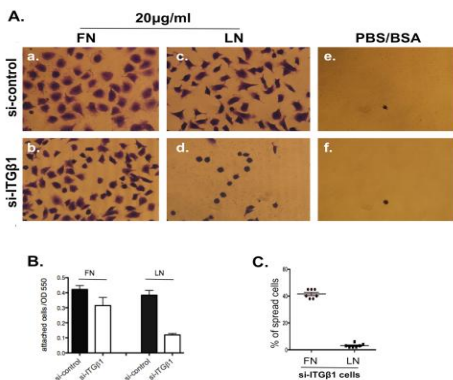

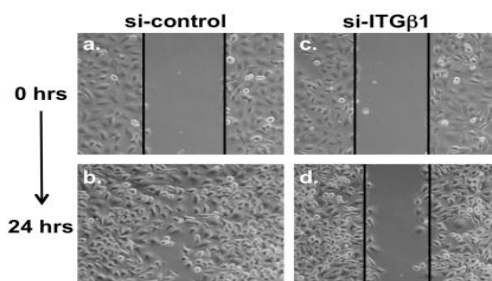**Figure 8. ITGβ1-dependent Hela cell adhesion.**

Figure 9. Integrin-dependent wound healing and migration of HeLa cells.

This difference was still obvious when cells were incubated even longer 120 minutes, indicating that ITGβ1 binding to laminin is crucial for HeLa cells attachment and spreading. As a negative control cells were plated onto dishes coated with PBS/BSA (Figure 8A-ef). The quantification analysis is shown in Figures 8B and 8C. Thus, ITGβ1 mediates cell shape adhesion and spreading in HeLa cells. Adhesion is a complex mechanism and involves variety processes like cell migration, invasion, wound healing and tissue remodeling [Wiesner at al., 2005; Legate at al., 2006]. The next results demonstrated that only in the presence of ITGβ1 cells are able to migrate and repair the wound (Figure 9ab) in contrast to si-ITGβ1 cells (Figure 9cd).

3. Vaccinia virus *WR53.5/F14.5* protein: a new component of intracellular MV:

Conserved Vaccinia *WR53.5/F14.5 ORF* encodes a late viral envelope protein in MV particles. MASS analysis of newly prepared Vaccinia vT7lacOI MV virions revealed two tryptic peptides, YVEENNEEDAR and IKEEQELLLLY, that had not been detected before and matched a small ORF *WR53.5* protein in Vaccinia virus[11]WR strain genome (Figure 10A), encoding a conserved polypeptide of 49 aa with a predicted molecular weight of 5.5 kDa

(http://www.poxvirus.org/). Hydropathy analysis predicted that the *WR53.5* protein has two hydrophobic domains at the N-and C-termini (Figure 10B). Alignment of the amino acid sequences of Vaccinia *WR53.5L* and its orthologues present in the *Orthopoxvirus* genus revealed a high level of homology among these proteins (~98% conserved residues) including the *F14.5L* ORF in Vaccinia Copenhagen strain [Goebel, Johnson et al. 1990]. The anti-53.5 antiserum that we obtained recognized a small 3.5 kDa viral protein in cells infected with our original WR strain Vaccinia virus. (*WR-1*) and a 5.5 kDa protein in cells infected with other Vaccinia strains including two WR strains (vT7lacO/I and WR-2), Copenhagen (Cop) and IHD-J strains (Figure 10C). Although WR and Cop strains have been sequenced before we re-sequenced the *WR53.5L* gene locus in the above virus genomes and found that the *WR53.5L* ORF in WR-1 specifies a K at residue 44, encoded by AAA, whereas an E encoded by GAA was found in other Vaccinia virus genomes such as vT7LacO/I, WR-2, Cop and IHD-J strains (Figure 10D), suggesting that a non-conserved G-to-A mutation occurred in the WR-1 genome, resulting in a glutamic acid-to-lysine change at position 44 and a faster electrophoretic mobility on SDS-PAGE gels prepared from vT7lacO/I and *viG3L* virus, a (Figure 10E). Since E44 was more frequently found than K44 in the different *WR53.5* orthologues (Figure 10B), we named that the WR53.5^{E44} in WR-2 as the wild-type *WR53.5* protein. Wild-type *WR53.5* protein was detected in MV particles in vT7LacO/I; however, the *WR53.5^{K44}* protein was barely detected in purified WR-1 MV (Figure 10E). Expression of the wild-type *WR53.5* protein was monitored in cells infected with Vaccinia virus WR-2, and a 5.5 kDa protein was detected at 4 h p.i, which increased in abundance until 24 h p.i. (Figure 10F).
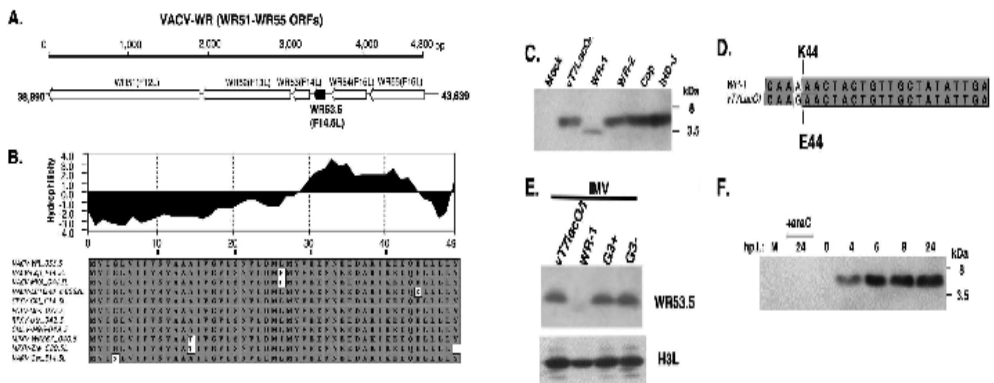

Figure 10. WR-53.5 ORF, conserved small protein and expresses late in an affected cells.

Plaque formation and MV/EEV production on cells infected with recombinant vi53.5 virus with/without IPTG: The role of *WR53.5L* during the Vaccinia virus life cycle in cell culture was explored using a recombinant Vaccinia virus, vi53.5L, that was generated from the vT7LacOI parental virus. vi53.5L contains an inducible *WR53.5L*/ *E. coli gpt* marker gene cassette inserted into its endogenous *WR53.5L* locus and[12]was isolated in the presence of mycophenolic acid and purified after 3 rounds of plaque purification. Immunoblot reveled, that

expression of the *WR53.5L* gene was tightly regulated at the late phase by IPTG. Further BSC40 cells were infected with vi53.5L in the presence or absence of IPTG and monitor at 3 days p.i., in the control vT7LacOI and vi53.5L with/without IPTG, similar plaque shape was observed and virus titer was similar as well, showing that *WR53.5* protein is not required for plaque formation and MV production. We thus concluded that *WR53.5* protein is not required for Vaccinia virus life cycle in cell cultures.
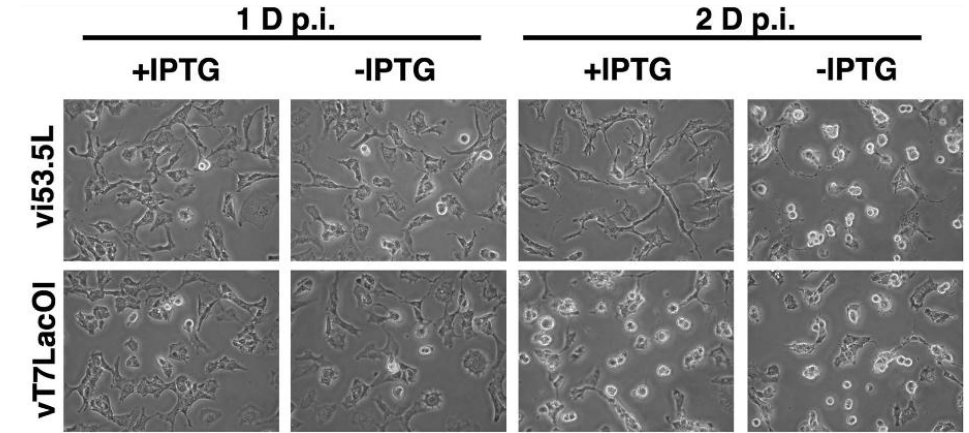

Figure 11. Morphology of BSC40 cells infected with vi53.5L virus in the absence and in the presence of IPTG.

The role of WR-53.5 protein in cell morphology and adhesion of virus-infected BSC40 cells: Interestingly, when BSC40 cells were infected with vi53.5L and maintained in IPTG-containing medium the morphology of virus-infected cells gradually changed into elongated shape at 1-2 days p.i. (Figure 11). In the absence of IPTG, the infected cells rounded up and became loosely attached to dishes at 2 days p.i., suggesting that expression of *WR-53.5* protein induced by IPTG affected cell morphology. However, the elongated morphology was not obvious in BSC40 cells infected with the parental virus vT7LacOI that expresses *WR-53.5* protein from its endogenous promoter and is not regulated by IPTG (Figure 11). All this demonstrated that a high level of *WR-53.5* protein expression allowed detection of its function in cell adhesion.

The role of *WR53.5* protein in Ca^{2+}-independent cell adhesion in BSC40 cells infected with vi53.5L virus: To test whether WR-53.5-mediated cell adhesion was dependent on calcium, we infected BSC40 cells with vi53.5L and treated the infected cells with EGTA (Figure 12). Mock-infected BSC40 cells adhered well to plates but rounded up after EGTA treatment. On the other hand, cells infected with vi53.5L, remained adherent even after EGTA treatment, showing that *WR-53.5* protein is required for Ca^{2+}-independent cell adhesion of the infected cells. The fact that *WR-53.5* mediates cell adhesion suggests a 13 possibility that it is expressed on the surface of virus-infected cells. As expected FACS analyses shown in (Figure 13), strong fluorescent staining

of *WR-53.5* protein is detected on BSC40 cells infected with vi53.5L in the presence of IPTG but not in the absence of IPTG and less strong staining in other virus strains.
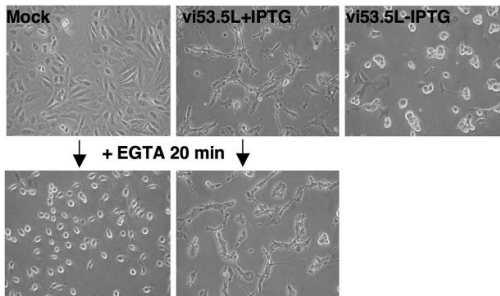

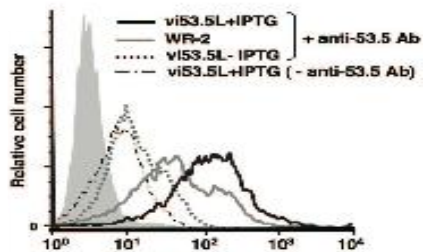**Figure 12. EGTA treatment of Mock or vi53.5 infected cells.**

Figure 13. Flow cytometry of WR-53.5 protein expression on infected cells.

In vivo examination of *WR53.5* protein: Although *WR-53.5* expression was tightly regulated by IPTG in vi53.5L virus, it is not suited for *in vivo* studies because its tk locus was inactivated by inserting a T7 RNA pol cassette [Ward et al., 1995]. We generated a deletion virus, *WRΔ53.5L*, that inactivated *WR-53.5L* ORF from wild type WR-2 Vaccinia virus (Figure 14A). And use for mice experiment as shown in (Figure 14B), all the mice infected by wild type WR-2 virus or *WRΔ53.5L* virus started losing weight at 4 days p.i., continued weight loss for another 5-7 days. Some infected mice were severely ill and died at day 11 whereas others slowly recovered. The difference of weight loss between mice infected by wild type WR-2 virus or *WRΔ53.5* virus was small in all three dosages (Figure 14B).
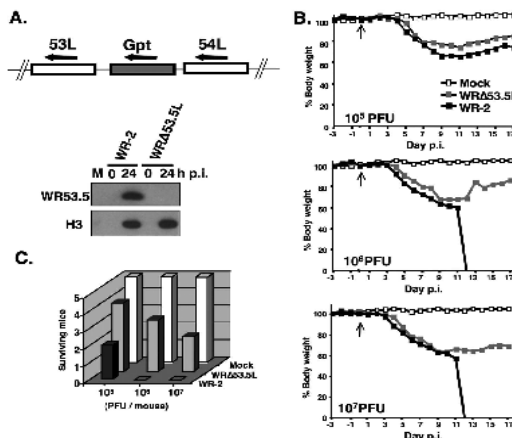

Figure 14. WR53.5 protein contributes to Vaccinia virus virulence in mice.

However, all the mice infected with 10^6 and 10^7 PFU wild type WR-2 virus, died by 12 days p.i., whereas 60% and 40% of mice infected by *WRΔ53.5L* mutant virus survived respectively

(Figure 14C). We thus concluded that WR53.5 protein contributes to Vaccinia virus virulence in vivo.

4. The role of envelope *G3L* protein in Vaccinia virus cell entry:

Conserved Vaccinia *G3L* gene encodes a late viral envelope protein in MV. Hydropathy analysis revealed two hydrophobic domains at N- and C-termini of *G3L* (Figure 15A). Alignment of Vaccinia *G3L* amino acid sequences and its orthologues in the poxvirus family (Figure 15B) revealed a high level of homology (46% conserved residues), suggesting that *G3L* might play important role in poxvirus life cycle. The internal region of *G3L* was less conserved than N- and C-terminal regions.

Anti-G3L antibody was generated and used to study the expression of *G3L* during Vaccinia virus infection. Rabbits were immunized with a synthetic peptide derived from the *G3L* amino acid sequence, and the antiserum produced was tested on immunoblots of lysates prepared from virus-infected cells. The antiserum did not recognize any protein in mock infected cells but recognized a 12.8-kDa protein in infected cells that was detected at 8 h p.i. and increased in abundance until 24 h p.i. (Figure 15C).
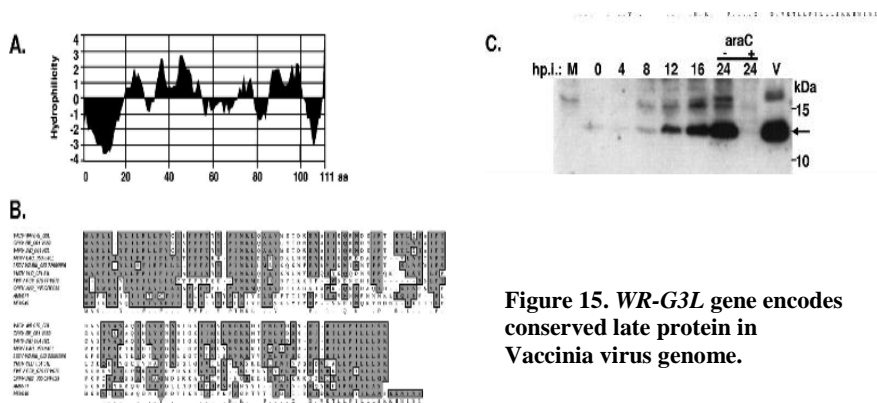

Figure 15. *WR-G3L* gene encodes conserved late protein in Vaccinia virus genome.

The 12.8-kDa protein was expressed at the late phase in virus-infected cells, since araC, which inhibits viral DNA replication, blocked *G3L* expression (Figure 15C).

The antiserum also recognized a 12.8-kDa protein in purified MV, demonstrating that *G3L* protein is present in MV particles.

Vaccinia *G3L* as an MV membrane component: Purified MVs were extracted and the virion membrane proteins were separated from the insoluble core components. As was expected, *G3L* was extracted from purified Vaccinia MV with 1% NP-40 inclusion of DTT during extraction did not result in greater release of *G3L* into the supernatant the results showed that *G3L* is associated with membranes. Another MV membrane protein, *H3L*, served as a control and was similarly extracted into the supernatant fraction. In[15]contrast, the viral core proteins 4a/4b were

resistant to detergent extraction. Thus, *G3L* is a late protein that is present in the membrane of Vaccinia MV.
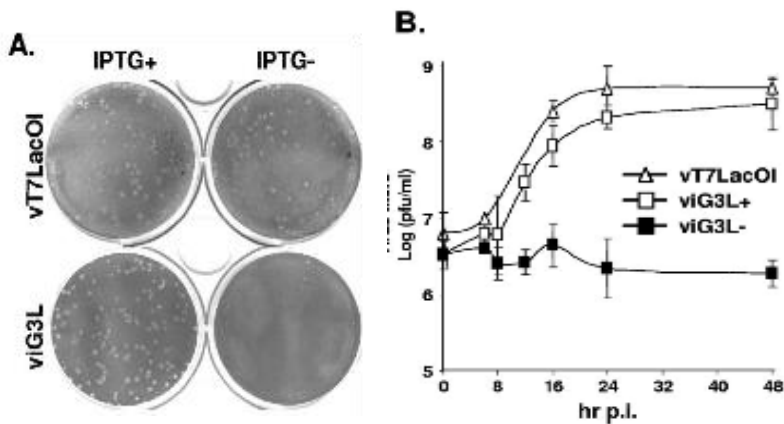

Figure 16. Plaque assay and one-step growth curve of viG3L virus in BSC 40 cells.

Construction of a recombinant virus with inducible *G3L* gene: Here as well to explore the role of *G3L* during Vaccinia virus life cycle in cell culture we generated a recombinant Vaccinia virus, viG3L, expressing *G3L* conditionally regulated by IPTG [Izmailyan et al., 2006] viG3L was generated from the vT7LacOI parental virus with an inducible *G3L* cassette that contains the E.coli gpt marker gene inserted into its endogenous locus. Then we tested G3L protein product in viG3L recombinant virus on BSC40 cells that were infected with or without IPTG, abundant *G3L* was only detected in the cells that were infected in the presence of IPTG, and its production was blocked by araC demonstrating that expression of the *G3L* gene is tightly regulated at the late phase by IPTG.

Plaque formation and mature virus production with or without *G3*L expression: The role *G3L* in BSC40 was investigated cells infected with viG3L in the presence or absence of IPTG demonstrated that the control parental virus, vT7LacOI, formed similar plaques on cells in the presence or absence of IPTG, whereas viG3L only formed plaques in the presence of IPTG, suggesting that *G3L* expression is required for plaque formation. (Figure 16A), The measurement of viG3L titer showed a 2-log increase at 24 and 48 h p.i., similar to the vT7lacOI parental virus, whereas viG3L grew poorly in the absence of IPTG, with no increase in titer at 24 or 48 h p.i. (Figure 16B), suggesting that *G3L* is required for Vaccinia virus growth in cell culture.

***G3L* is not required for virion morphogenesis of MV and EV:** The study of MVs produced in viG3L infected BSC40 cells in the presence or absence of IPTG revealed that viral crescents, immature virions and other intermediate membrane structures are detected at 12 h.p.i., and a large number of dense mature MV particles are detected in the cytoplasm at 24 hp.i. These MV particles in both cells appeared to be [16]indistinguishable from each other suggesting
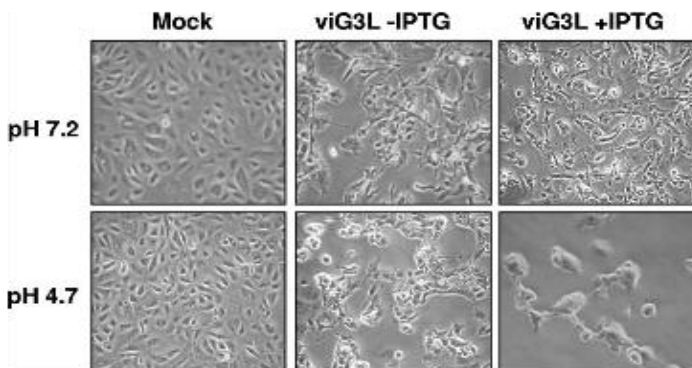
that *G3L* is not required for MV formation**.**

**Figure 17. G3L imediates virus infected cell-cell fusion in a
low-pH dependant manner**

        Involvement of *G3L* for cell-cell fusion induced by low-pH treatment. The study of the *G3L* in BSC-1 cell fusion from within showed that viG3L infected cells in the presence of IPTG do not develop cell fusion at neutral pH at 24 h.p.i. However, they developed into gigantic fused cells in acidic conditions. In the absence of IPTG when *G3L* protein is not expressed no cell fusion is observed suggesting that *G3L* protein is required for low-pH-triggered cell–cell fusion (Figure 17),

CONCLUSIONS

1. It is shown for the first time that lipid raft protein integrin β1 is important for Vaccinia virus entry. It associates with Vaccinia virus mature particles in lipid raft microdomain and is mediates both Vaccinia virus attachment and penetration steps.
2. PI3K/Akt signaling is crucial for Vaccinia virus entry into cells and is dependant from integrin β1. Vaccinia virus mature particles bind to the cells and activate PI3K/Akt phosphorilation. Moreover, pharmocological inhibitors of PI3K (LY294002) and/or Akt (Akt IV) reduce Vaccinia virus infection in both MEF and HeLa cells.
3. Newly discovered Vaccinia *WR-53.5* protein is expressed abundantly on the surface of infected cells and regulates cell morphology and adhesion. Attachment phenotype of *WR53.5* protein is Ca2+-independent.
4. *WR53.5* contributes to Vaccinia virus virulence in vivo.
5. Vaccinia *WR-G3L* gene is conserved encodes a late viral envelope protein and presents in the membrane fraction of Vaccinia mature virion.
6. Vaccinia virus *WR-G3* protein is essential[17] for virus entry, plaque formation, mature virus production and virus penetration into cells, mediates low-pH-triggered cell-cell

fusion and is an essential component of entry fusion complex.

PUBLICATIONS RELEVANT TO THESIS
Articles

1. Izmailyan R. Integrin β1 requires for adhesion spreading and migration of HeLa cells. // Biological Journal of Armenia. 2013; 3(65): 6-10.

2. Izmailyan R., Hsao J.C., Chung C.S., Chen C.H., Hsu P.W-C., Liao C-L., Chang W. Integrin β1 mediates vaccinia virus entry through activation of PI3K/Akt signaling. // Journal of Virolology. 2012; 86(12): 6677-6687.

3. Chang S-J., Chang Y-X., Izmailyan R., Tang Y-L., Chang W. Vaccinia virus A25 and A26 proteins are fusion suppressors for mature virions and determine strain-specific virus entry pathways into HeLa, CHO-K1, and L cells. // Journal of Virology. 2010; 84(17): 8422-8432.

4. Izmailyan R., Chang W. Vaccinia virus WR53.5/F14.5 protein is a new component of intracellular mature virus and is important for calcium-independent cell adhesion and vaccinia virus virulence in mice. // Journal of Virology. 2008; 82(20): 10079-10087.

5. Izmailyan R.A., Huang C-Y., Mohammad S., Isaacs S.N., Chang W. The envelope G3L protein is essential for entry of vaccinia virus into host cells. // Journal of Virology. 2006; 80(17): 8402-8410.

Conference materials

1. Izmailyan R., Hsao J.C., Chung C.S., Chen C.H., Hsu P.W., Liao C.L., Chang W. Integrin beta 1 mediates Vaccinia virus entry through activation of PI3K/AKT signaling. // Abstract book of the 19th International Poxvirus and Iridovirus Conference, Salamanca, Spain, 2012, p. 60.

2. Izmailyan R., Chang W. Vaccinia viral protein WR53.5/F14.5 is a new component of intracellular mature virus and is important for calcium-independent cell adhesion and vaccinia virus virulence in mice. // Abstract book of the 28th Annual Meeting of American Society for Virology, British Columbia Vancouver, Canada, 2009, p. 267.

3. Izmailyan R., Chang W. Vaccinia virus WR53.5L is an envelope protein on intracellular mature virions that mediates calcium-independent cell adhesion and vaccinia virus virulence *in vivo*. // Abstract book of the 17th International Poxvirus and Iridovirus Conference, Grainau (Bavaria), Germany, 2008, p. 42.

4. Izmailyan R., Huang C.Y., Isaacs S.N., Chang W. The envelope G3L protein is essential for entry of Vaccinia virus into host cells. //18   Abstract book of the FASEB Summer Research Conference, Indian Wells,   California, 2006, p. 41.

5. Chung C.S., Huang C.H., <u>Izmailyan R.</u>, Chao D.Y., Ching Y.C., Tang Y.L., Chang W. Virus entry and host cell signaling. // Abstract book of the conference "Mol. Bio. in the 21th Century: Interface, Integration, and Perspectives", Taipei, Taiwan, 2006, p.78.

Իզմահլյան Ռոզա Արտաշեսի

ՎԱԿՑԻՆԻԱ ՎԻՐՈՒՍԻ ԲՋԻՋ ՆԵՐԹԱՓԱՆՑՄԱՆ ՈՒՂԻՆԵՐԻ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ

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Առանցքային բառեր. *ինտեգրին β1, G3L վիրուսային սպիտակուց, WR-53.5 վիրուսային սպիտակուց, Վակցինիա վիրուս, տեր-վիրուս փոխազդեցություն:*

Վակցինիա վիրուսը պատկանում է *Poxviridae* ընտանիքի *Orthopoxvirus* դասին: *Poxviridae* ընտանիքին պատկանող վիրուսներն այլ կենդանիների վիրուսներից տարբերվում են իրենց մեծ չափերով, բարդ կառուցվածքով և ունեն վարակելու յուրահատուկ լայն շրջանակներ՝ վարակելով գրեթե բոլոր բջջային կուլտուրաները *in vitro*, ինչպես նաև մարդկանց և կենդանիներին *in vivo*: Այս ընտանիքին պատկանող ամենակարևոր և ամենահայտնի անդամն է Ծաղկի վիրուսը, որը կոչվում է նաև Վարիոլա կամ Սմուլփոքս և հանդիսանում է մարդկանց մոտ ծաղիկ հիվանդության հարուցիչ: Հայտնի է, որ հազարամյակներ շարունակ Ծաղկի վիրուսը խլել է միլիոնավոր մարդկանց կյանքեր, սակայն դեռևս լիովին բացահայտված չեն ինչպես դրա առաջացման, այնպես էլ մարդկանց փոխանցվելու ուղիները:

1980 թվականին Առողջապահության Համաշխարհային Կազմակերպությունը (WHO) հայտարարեց, որ ծավալուն պատվաստումների շնորհիվ Ծաղկի վիրուսը ոչնչացվել է բնական միջավայրից, որի արդյունքում դադարեցվեցին դրա դեմ պատվաստումները: Սակայն այդ վիրուսների ուսումնասիրությունն առ այսօր մնում է արդիական և կարևոր՝ հաշվի առնելով այն փաստը, որ Ծաղկի վիրուսի նմուշի բնօրինակը դեռևս պահպանվում է Ռուսաստանի Դաշնության և Ամերիկայի Միացյալ Նահանգների որոշ լաբորատորիաներում և կարող է հանդիսանալ որպես կենսաբանական զենք: Բացի այդ, հայտնի են նաև կենդանուց մարդուն փոխանցման այլ փոքսվիրուսների հարուցած ինֆեկցիաների դեպքեր: Օրինակ՝, կապիկի փոքսը փոխանցվելով մարդուն, կարող է առաջացնել ծաղկի վարակի խմբին հատուկ հիվանդություն: Հետևաբար, մեծ է վտանգը, որ ժամանակի ընթացքում կապիկի փոքսը կարող է փոխարինել Ծաղկի վիրուսին և լուրջ համաճարակի խթան հանդիսանալ: Այդ պատճառով շատ կարևոր է ուսումնասիրել Վակցինիա վիրուսի ռեպլիկացիայի և ներթափանցման ուղիների մեխանիզմները:

Վակցինիա վիրուսը թաղանթապատ, մոտ 200Կբ երկարությամբ երկշղթա ԴՆԹ-ով վիրուս է, որը կոդավորում է ավելի քան 200 բաց ընթերցման շրջանակներ: Բջիջ ներթափանցելուց հետո վիրիոնի մորֆոգենեզը տեղի է ունենում

բացառապես ցիտոպլազմում՝ արտադրելով մի քանի տեսակի վարակիչ մասնիկներ, մասնավորապես,՝ հասուն վիրուս, որը բաղկացած է մեծ թվով սպիտակուցներից, որոնք էլ նպաստում են վիրուսի ներթափանցման բարդ գործընթացներին, ինչպես նաև թաղանթապատ վիրուս և բջջից դուրս գտնվող վիրուս:

Մեր լաբորատորիայում ստացված նախորդ արդյունքները ցույց են տվել, որ բջջի թաղանթում գտնվող լիպիդային ռաֆտերը բարենպաստ հարթակ են հանդիսանում հասուն վիրուսի ներթափանցման համար:

Տվյալ աշխատանքում Վակցինիա վիրուսով վարակված HeLa բջիջների լիպիդային ռաֆտերի ուսումնասիրությունը ցույց է տվել, որ վիրուսի արդյունավետ ներթափանցման գործընթացի ժամանակ բազմաթիվ բջջային սպիտակուցներ ենթարկվում են դինամիկ փոփոխությունների, և դրանցից 16%-ը մեզ հետաքրքրող բջջաթաղանթի սպիտակուցներն են՝ ինտեգրին β1-ը և դրա հետ ասոցացված մոլեկուլները: Վակցինիա վիրուսի հետ ինտեգրին β1-ի ասոցիացիայի ուսումնասիրությունը ցույց է տվել, որ այդ սպիտակուցը կարևոր դեր է խաղում Վակցինիա վիրուսի ներթափանցման գործընթացում ինչպես HeLa բջիջների, այնպես էլ մկան սաղմնային ֆիբրոբլաստների մոտ: Ավելին, ցույց է տրվել, որ Վակցինիա վիրուսի ինֆեկցիան հանգեցնում է ֆոսֆատիդիլինոզիտոլ-3-կինազի (PI3K) ներբջջային ազդակի ինտեգրին β1-կախյալ ակտիվացմանը:

Այսպիսով, առաջին անգամ հայտնաբերվել է բջջային ռեցեպտոր Վակցինիա վիրուսի համար և ապացուցվել է, որ Վակցինիա վիրուսի ասոցիացիան β1-ի հետ հանգեցնում է ֆոսֆատիդիլինոզիտոլ-3-կինեզի ակտիվացմանը, ինչն էլ նպաստում է վիրուսի էնդոցիտոզին HeLa բջիջներ:

Տվյալ աշխատանքում, հայտնաբերվել և ուսումնասիրվել են նաև երկու նոր հասուն Վակցինիա վիրուսի սպիտակուցներ՝ WR-53.5/F14.5 և WR-G3, որոնց ֆունկցիաններն և դերը Վակցինիա վիրուսի կյանքի ցիկլում դեռևս ուսումնասիրված չեն: Մեր կողմից կատարված ուսումնասիրությունները ցույց են տվել, որ WR-53.5/F14.5 սպիտակուցը կարևոր չէ վիրուսի ցիկլի համար բջջային կուլտուրաներում, սակայն նպաստում է վարակված բջիջների ադհեզիային և վիրուլենտությանը: Ավելին, մկների հետ կատարված ուսումնասիրությունները ցույց են տվել, որ առանց WR-53.5/F14.5 սպիտակուցի Վակցինիա վիրուսի վիրուլենտությունը կտրուկ նվազում է: Եվ վերջապես, WR-G3 սպիտակուցի հետազոտությունը ցույց է տվել, որ այն կարևոր դեր է խաղում բջջային կուլտուրաներում Վակցինիա վիրուսի կյանքի ցիկլի համար:

Այսպիսով, Վակցինիա վիրուսի հետ ասոցիացված բջջային ռեցեպտորների և վիրուսային նոր սպիտակուցների բացահայտումը ոչ միայն ընդլայնում և լրացնում է Վակցինիա վիրուսի ներթափանցման գործընթացների մոլեկուլա-բջջային մեխանիզմների վերաբերյալ առկա գիտելիքները, այլ նաև նպաստում է Վակցինիա վիրուսի կանխարգելման, նոր հակավիրուսային միջոցների ստեղծման, ինչպես նաև վարակված անհատների[20]բուժման նոր մոտեցումների մշակմանը:

Измаилян Роза Арташесовна

ИССЛЕДОВАНИЕ ПУТЕЙ ПРОНИКНОВЕНИЯ ВИРУСА ВАКЦИНИИ В КЛЕТКУ

Р Е З Ю М Е

Ключевые слова - *интегрин β1, вирусный белок G3L, вирусный белок WR-53.5, вирус Вакцинии, взаимодействие вируса с хозяином.*

Вирус Вакцинии принадлежит семейству *Poxviridae*, классу *Orthopoxvirus*. Вирусы семейства *Poxviridae* отличаются от других животных вирусов большими размерами, структурными сложными характеристиками и заражают почти все клеточные культуры *in vitro*, а так же людей и животных *in vivo*. Самый важный и самый известный вирус, принадлежащий к этому семейству – это вирус оспы который нам известен так же под названием Вариола или Смолпокс и является возбудителем болезни оспы у людей. Несмотря на то, что вирус оспы очень древний вирус и в течении тысячелетий отнял жизни у миллионов людей, до сих пор полностью не иследованы как происхождение данного вируса, так и пути его передачи человеку.

В 1980 году Всемирная организация здравоохранения объявила, что благодаря масштабным прививкам вирус оспы уничтожен из естественной среды, после чего были прекращены прививки против оспы. Тем не менее, исследование вирусов группы оспы по-прежнему актуально и важно по следующим причинам: образец вируса до сих пор хранится в некоторых лабораториях Российской Федерации и США и по сей день является образцом биологического оружия. Кроме того известны случаи, когда инфекции, возбуждаемые другими поксвирусами, могут быть переданы человеку от животного. Например, вирус обезьянней оспы, передаваясь человеку, вызывает болезнь, похожую на оспу. Следовательно, велика опасность, что со временем вирус обезьянней оспы может заменить натуральную оспу и вызвать серьезную эпидемию. Поэтому, учитывая вышеизложенное, исследование механизмов путей проникновения и репликации данного вируса являются важной задачей для ученных данной сферы.

Вирус Вакцинии - это оболочечный вирус с двухцепочной ДНК величиной ~ 200кб и кодирует более чем 200 открытые рамки считывания. После проникновения в клетку морфогенез вируса происходит исключительно в цитоплазме, выделяя заражающие частицы нескольких видов, а именно, зрелый вирус, состоящий из большого количества белков, способствующих сложному процессу проникновения вируса, а так же вирус покрытый оболочкой и внеклеточный вирус.

Предыдущие результаты, полученные в нашей лаборатории показали, что липидные рафты, находящиеся в оболочке клетки, являются платформой для проникновения

зрелого вируса. В данной работе исследования липидных слоев клеток HeLa, зараженных вирусом Вакцинии, показали, что в процессе удачного проникновения вируса многие клеточные белки подвергаются динамическим изменениям. В ходе дальнейших исследований было выявлено, что 16% из этих белков являются интересующие нас белки клеточный мембраны - интегрин β1 и ассоцирующие с ним молекулы. Исследование связи интегрина β1 с вирусом Вакцинии показало, что данный белок играет важную роль в проникновении вируса Вакцинии как в клетки HeLa, так и в мышиные эмбриональные фибробласты. Более того, было показано, что инфекция вируса Вакцинии приводит к интегрин β1-зависимой активации фосфатидилинозитол-3-киназа.   Таким образом, в данной работе впервые обнаружен клеточный рецептор для вируса Вакцинии и было доказано, что ассоциация вируса Вакцинии с интегрином β1 приводит к активизации фосфатидилинозитол-3-кинеза, что способствует эндоцитозу вируса в клетку HeLa.

Были также обнаружены и исследованы два новых белка зрелых вирусов Вакцинии - *WR-53.5/F14.5 и WR-G3*, функция и роль которых в цикле жизни вируса Вакцинии не были ранее исследованы. Исследование показало, что белок WR-*53.5/F14.5* не важен для цикла вируса в клеточных культурах, однако способствует адгезии зараженных клеток и вирулентности. Более того, эксперимент на мышах показал, что когда белок *WR-53.5/F14.5* не производится, вирулентность вируса Вакцинии резко понижается. И наконец, исследование белка *WR-G3* показало, что этот белок играет важную роль в цикле жизни вируса Вакцинии в клеточных культурах.

Таким образом, выявление клеточных рецепторов и белков вируса Вакцинии не только расширяет и обогащает существующие знания относительно молекулярно-клеточных механизмов проникновения вируса, но и способствует разработке новых противовирусных препаратов, а так же новых подходов для предотвращения заражения и для лечения уже зараженных пациентов.