UNIVERSITI PUTRA MALAYSIA

CLATHRIN- AND CAVEOLAE- INDEPENDENT ENDOCYTOSIS OF NEWCASTLE DISEASE VIRUS STRAIN AF2240 INTO HELA CANCER CELL LINE

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By

NURHAZWANI BINTI SUKRAM

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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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June 2014

Chairman : Associate Professor Norazizah Binti Shafee, PhD

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Newcastle disease virus (NDV), a *Paramyxoviridae,* is an enveloped virus with a single stranded, non-segmented negative sense RNA genome. Studies on oncolytic activity of certain strain of NDV have yielded encouraging results and increased the interest of researcher to develop it as cancer therapy. Despite the interest, the exact mechanism of how the virus induces oncolysis is not known and the process of its entry into cells is also not fully understood. To understand the entry process, the present study was designed with the main objective to determine the endocytic pathway, mainly receptor mediated, of a velogenic local strain of NDV strain AF2240 into HeLa cancer cells. The objectives were divided into three parts, which are, to evaluate the cytotoxicity effects of chlorpromazine (CPZ) and genistein on HeLa cell viability; to determine the effect of chlorpromazine (CPZ) and genistein on NDV nucleocapsid protein (NP) expression; and to study the involvement of caveolin-1 protein in NDV AF2240 entry into HeLa cells. It was hypothesized that the NDV enter the cells via caveolae-mediated endocytosis, and caveolin-1 protein will be involved in the process. Data from the study showed that the IC_{50} of CPZ is 5.829 (± 0.075) μM and as for genistein, the IC_{50} is above 500 μM. It was found that in dose- and time- dependent manner, CPZ does not cause any effect to the NDV NP expression, whereas genistein inhibits the NP expression at the concentration of 250 μM and at 3 h p.i with the same concentration. However, the data obtained from confocal analysis showed that the internalization of NDV into HeLa cells might not be mediated by caveosomes (a caveolin-coated endocytic vesicle) since there is not caveolin-1 found when NDV internalized into the cells. This data suggested NDV AF2240 might deploy an alternative method of entry, which is clathrin-independent, cholesterol-dependent endocytosis that is not dependent on the presence of caveolin-1. Information regarding these steps in viral entry would shed light into understanding the virus mechanism towards cancer cell killing mechanism. These might help in the rational design for NDV oncolytic studies.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

ENDOSITOSIS BEBAS *CLATHRIN* DAN *CAVEOLAE* OLEH VIRUS NEWCASTLE DISEASE DARI STRAIN AF2240 KE ATAS SEL KANSER HELA

Oleh

NURHAZWANI BINTI SUKRAM

Jun 2014

Pengerusi : Profesor Madya Norazizah Binti Shafee, PhD

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Virus Newcastle Disease (NDV), sejenis *Paramyxoviridae*, ialah virus bersampul yang mempunyai genom RNA beruntai tunggal dengan rantaian negative tanpa segmen. Kajian ke atas aktiviti onkolitik pada beberapa strain NDV telah membuahkan hasil yang menggalakkan dan telah menarik minat ramai penyelidik untuk menjadikan NDV sebagai terapi kanser. Namun begitu, mekanisme sebenar tentang bagaimana virus ini menyebabkan *oncolysis* berlaku masih tidak diketahui dan proses kemasukan virus ini ke dalam sel juga masih tidak difahami sepenuhnya. Oleh itu, kajian ini telah dijalankan dengan objektif utama untuk menentukan laluan secara endositosis oleh NDV dari stain AF2240 ke dalam sel HeLa. Objektif kajian ini dibahagikan kepada tiga iaitu; menentukan kadar *cytotoxicity* oleh chlorpromazine (CPZ) dan genistein ke atas sel HeLa; menentukan efek chlorpromazine (CPZ) dan genistein terhadap ekspresi protein *nucleocapsid* (NP) NDV; dan melihat penglibatan protein *caveolin-1* dalam kemasukan NDV AF2240 ke dalam sel HeLa. Hipotesis menyatakan bahawa NDV memasuki sel melalui jalan lintasan *caveolae*, di mana protein *caveolin-1* terlibat di dalam proses tersebut. Data yang diperolehi menunjukkan IC_{50} bagi CPZ ialah 5.829 (± 0.075) μM, manakala IC_{50} bagi genistein melebihi 500 μM. CPZ didapati tidak memberi kesan pada penghasilan protein NP, akan tetapi, genistein memberi efek pengurangan dalam penghasilan protein NP apabila genistein digunakan pada 250 μM dan juga selepas 3 jam infeksi terhadap NDV. Namun begitu, penglibatan *caveolin-1* di dalam proses kemasukan NDV didapati negatif, dan membawa kepada ketiadaan *caveosomes* dalam membantu kemasukan NDV ke dalam sel HeLa. Kesimpulan yang diperolehi dari kajian ini membawa kepada kaedah lain yang mungkin digunakan oleh NDV dalam proses kemasukan ke dalam sel, yang tidak melibatkan *clathrin*, tetapi melibatkan kolestrol dan tidak bergantung kepada *caveolin-1*. Maklumat mengenai langkah-langkah yang terlibat dalam proses kemasukan virus ini ke dalam sel kanser berpotensi dari segi pemahaman mengenai mekanisme virus terhadap pembunuhan sel kanser. Ini secara tidak langsung dapat membantu bagi membentuk satu kajian untuk bidang *oncolytic* menggunakan NDV.

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I certify that a Thesis Examination Committee has met on 16 June 2014 to conduct the final examination of Nurhazwani Binti Sukram on her thesis entitled "Clathrin- and Caveolae- Independent Endocytosis of Newcastle Disease Virus Strain AF2240 into HeLa Cancer Cell Line" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

%	percentage
°C	degree Celsius
µl	micro-liter
µM	micro-molar
BCA	bicinchoninic acid
BSA	bovine serum albumin
CO^2	carbon dioxide
CPZ	Chlorpromazine
DAPI	4', 6-diamidino-2-phenylindole, dihydrochloride
ddH_2O	double distilled water
dH_2O	distilled water
DiD	1, 1′-dioctadecyl-3, 3, 3′, 3′-tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
ECL	enhanced chemilumonescence
EDTA	ethylene diaminetetraacetate
F	fusion (protein)
FBS	fetal bovine serum
FITC	fluorescein isothiocyanate
g	gravity
genistein	genistein (4',5,7,-trihydroxyisoflavone)
h	hour
HA	hemagglutination
HeLa	human cervical cancer cells
HCl	hydrochloric acid
KCl	potassium chloride
KH_2PO_4	potassium dihydrogen phosphate
L	large (protein)
M	matrix (protein)
mg	milligram
min	minute
ml	milliliter
mM	millimolar
MOI	multiplicity of infection
MTT	3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
NaCl	sodium chloride
$NaHPO_4$	monosodium phosphate
NaOH	sodium hydroxide
ND	Newcastle disease
NDV	Newcastle disease virus
nm	nanometer
NP	nucleocapsid (protein)
OD	optical density

P	phosphoprotein
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
pH	*puissance hydrogen*
PI	propidium iodide
RIPA buffer	radioimmunoprecipitation assay buffer
RNA	ribonucleic acid
ROI	region of interest
rpm	revolutions per minute
RBC	red blood cell
s	seconds
SDS	sodium dodecyl sulfate
SW620	human colorectal adenocarcinoma cell line
TAE	tris-acetate-EDTA
TBS	tris- buffer saline
TBST	tris- buffer saline with Tween 20
TEMED	N, N, N'N'-tetramethylethylenediamine
TO	thiazole orange
Tris	tris(hydroxymethyl)aminomethane
UV	ultraviolet
V	volt
v/v	volume per volume
w/v	weight per volume

CHAPTER 1

INTRODUCTION

Newcastle disease (ND) is a contagious viral infection causing respiratory and nervous disorders in several avian species including chickens, quail and turkeys. This disease usually leads to acute and sudden death to many birds in the infected flock. In Malaysia, this disease is known as 'penyakit sampar ayam'. The virus causing this disease is known as Newcastle disease virus (NDV) and it also causes mild conjunctivitis and laryngitis in humans. It belongs to the genus *Avulavirus*, in family *Paramyxoviridae* (Mayo, 2002). Therefore, it is an enveloped virus with a single stranded, non-segmented negative sense RNA genome of approximately 15,000 nucleotides in length (Kolakofsky *et al.*, 1974).

As obligate intracellular parasites (Sieczkarski & Whittaker, 2005), viruses need to exploit the host cell machinery for their replication and propagation (Beer *et al*., 2005). Virus entry is initiated through the recognition by receptors present on the surface of the host cells. These receptors will act as mediators for viral tropism, and the receptor interactions occur in a programmed series of events utilizing multiple receptors (Chazal & Gerlier, 2003; Sieczkarski & Whittaker, 2005). The virus then must deliver its genome into the cytoplasm directly at the cell surface, by penetrating the plasma membrane, or after endocytosis by penetrating membranes of intracellular organelles such as the endosome for the infection to proceed (DeTulleo & Kirchhausen, 1998; Sieczkarski & Whittaker, 2005). However for enveloped viruses like NDV, they have been identified to use both direct fusion and endocytic pathway to penetrate the cells (San Roman *et al*., 1999; Beer *et al*., 2005; Cantin *et al*., 2007).

The endocytic pathways used by animal viruses to enter host cells include macropinocytosis, phagocytosis, clathrin- and caveolae-pathways and also non-clathrin, non-caveolae-dependent pathways (Swanson & Watts, 1995; Bishop, 1997; Aderem & Underhill, 1999; Marsh & McMohan, 1999; Sieczkarski & Whittaker, 2002; Damm *et al*., 2005; Cantin *et al*., 2007; Ghigo *et al*., 2008; Ivanov, 2008). Although it is already suggested previously that the paramyxoviruses, like NDV, fuse directly with the plasma membrane, it has also been suggested that NDV might also penetrate the cell by caveolae-mediated endocytosis (Alexander, 1999; San Roman *et al*., 1999; Cantin *et al*., 2007). However, mechanisms of NDV to invade targeted cells are still a matter of controversy.

In the past, a standard method for determining the route of viral entry is by direct electron microscopy at the early points after infection. However, this method requires a very high multiplicity of infection (MOI) of virus to ensure the particles can be located under the microscope, which leads to the tendency of visualizing viruses bound to non-productive sites as well as to their specific cellular target location (DeTulleo & Kirchhausen, 1998).

Studies on oncolytic activity of certain NDV strains have yielded encouraging results increasing the interest of using NDV vaccine for cancer therapy (Cassell & Garret, 1965; Reichard *et al*., 1992; Galili & Ben-Nathan, 1998; Schirrmacher *et al*., 2000; Fabian *et al*., 2001; Yusoff & Tan, 2001; Elankumaran *et al*., 2006). However, the methods of its entry are still questionable. Thus, it is inevitable to conduct a study on

NDV entry into cancer cells. A complete understanding of its intracellular trafficking may provide a rational design for cancer therapy.

In this study, attempts were made to study the endocytic entry of Newcastle Disease Virus (NDV) into cancer cell line. Cantin and team (2007) has provided an important clue to this study, where they have eliminated the clathrin endocytosis from the list of possible endocytic pathway for NDV. It is hypothesized that, if the NDV enter the cells via caveolae mediated endocytosis, and then caveolin-1 protein will be involved in the process. However, if there is no involvement of caveolin-1 in NDV entry, caveolae is not the route used by NDV to enter the cells. Hence, a local strain of NDV, AF2240 was used in this study to determine the endocytic pathway of NDV into the cancer cell. Although NDV was found to be infective against a few cancer cell lines, HeLa cells were chosen because, according to Cassel and Garrett, NDV was found to be active against this cell (Cassel & Garrett, 1965).

The main objective of this study is to investigate the endocytic mechanism of Newcastle disease virus (NDV) strain AF2240 against cancer cell lines. This study was undertaken with the following specific objectives:

1. To evaluate the cytotoxicity effects of specific pathway inhibitors, chlorpromazine (CPZ) and genistein, on HeLa cell viability.
2. To determine the effect of chlorpromazine (CPZ) and genistein on NDV nucleocapsid protein (NP) expression.
3. To study the involvement of caveolin-1 protein in NDV AF2240 entry into HeLa cells.

REFERENCES

Aderem, A., and Underhill, D. M. 1999. Mechanisms of phagocytosis in macrophages. *Annual Revision of Immunology* 17: 593–623.

Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M. and Fukami, Y. 1987. Genistein, a specific inhibitor of tyrosine-specific protein kinases. *Journal of Biological Chemistry*, 262(12): 5592-5595.

Alexander, D.J. 1997. Newcastle disease and other avian *Paramyxoviridae* infection. In; B.W. Calneck (Ed), Disease of the Poultry 10th edition. Ames, IA: Iowa State University Press pp 541-569.

Anderson, R.G.W. 1993. Caveolae: where incoming and outgoing messengers meet. Proceeding National Academy Science USA, Vol.90 pp.10909-10913.

Ballagi-Pordfiny, A., Wehmann, E., Herczeg, J., Belfik, S., and Lomniczi, B. 1996. Identification and grouping of Newcastle disease virus strains by restriction site analysis of a region from the F gene. *Archives of virology*, 141: 243-261.

Barnes, S., Kim, H., DarleyUsmar, V., Patel, R., Xu, J., Boersma, B. and Luo, M. Beyond ER alpha and ER beta: estrogen receptor binding is only part of the isoflavone story. *Journal of Nutrition*, 130: 656S-657S.

Baumann, C.A. and Neubert, W.J. 2010. Neuraminidase-deficient Sendai virus HN mutants provide protection from homologous superinfection. *Archives of Virology*, 155(2): 217-227.

Beer, C., Andersen, D.S., Rojek, A., and Pedersen, L. 2005. Caveolae-dependent endocytic entry of amphotropic Murine Leukemia virus. *Journal of Virology*, 79: 10776-10787.

Bishop, N.E. 1997. An update on non-clathrin coated endocytosis. *Reviews in Medical Virology* 7: 199-207.

Calder, P.C., and Yaqoob, P. 2007. Lipid Rafts - composition, characterization, and controversies. American Society for Nutrition. *Journal of Nutrition*. 137: 545–547.

Cantin, C., Holguera, J., Ferreira, L., Villar, E., and Barroso, I.M. 2007. Newcastle disease virus may enter cells by caveolae-mediated endocytosis. *Journal of General Virology*, 88: 559-569.

Cassell, W. and Garret, R. E. 1965. Newcastle disease virus is an antineoplastic agent. *Cancer*, 18: 863-868.

Chambers, P., Millar, N.S., Bigham, R.W., and Emmerson, P.T. 1986. Molecular cloning of complementary DNA to Newcastle disease virus and nucleotide

sequence analysis of the junction between the genes encoding the haemagglutination-neuraminidase and the large protein. *Journal Genetic Virology* 67: 475-486.

Chazal, N. and Gerlier, D. 2003. Virus entry, assembly, budding and membrane rafts. *Microbiology and Molecular Biology Reviews*, 67(2): 226-237.

Chen, Y. and Norkin, L.C. 1999. Extracellular Simian virus 40 transmits a signal that promotes virus enclosure within caveolae. *Experimental Cell Research* 246: 83-90.

Chia, S.L., Tan, W.S., Yusoff, K. and Shafee, N. 2012. Plaque formation by a velogenic Newcastle disease virus in human colorectal cancer cell lines. *Acta Virologica*, 56(4): 345-347.

Crennell, S.J., Takimoto, T., Portner, A. and Taylor, G. 2000. Crystal structure of the multifunctional paramyxovirus hemagglutininneuraminidase. *Nature Structure of Biology*, **7**: 1068–1074.

Csatary, L.K., Csatary E., and Moss R.W. 2000. Re: Scientific interest in Newcastle disease virus is reviving. *Journal of National Cancer Institute*, 92: 493-494.

Damm, E.M., Pelkmans, L., Kartenbeck, J., Mezzacasa, A., Kurzchalia, T. and Helenius, A. 2005. Clathrin- and caveolin-1- independent endocytosis: entry of simian virus 40 into cells devoid of caveolae. *Journal of Cell Biology*, 168: 477-488.

DeTulleo, L. and Kirchhausen, T. 1998. The clathrin endocytic pathway in viral infection. *The EMBO Journal* 17: 4585-4593.

Dixon, R.A. and Ferreira, D. 2002. Genistein. *Phytochemistry*, 60(3): 205-211.

Elankumaran, S., Rockemann, D. and Samal, S.K. 2006. Newcastle disease virus exerts oncolysis by both intrinsic and extrinsic caspase-dependent pathways of cell death. *Journal of Virology*, 80(15): 7522-7534.

Fabian, Z., Torocsik, B., Kiss, K., Csatary, L.K., Bodey, B., Tigyi, J., Csatary, C. and Szeberenyi, J. 2001. Induction of apoptosis by a Newcastle disease virus vaccine (MTH-68/H) in PC12 rat phaeochromocytoma cells. *Anticancer Research* 21: 125-136.

Ferguson, R.E., Carroll, H.P, Harris, A., Maher, E.R., Selby, P.J. and Banks, R.E. 2005. Housekeeping proteins: a preliminary study illustrating some limitations as useful references in protein expression studies. *Proteomics*, 5(2): 566-571.

Fiola, C., Peeters, B., Fournier, P., Arnold, A., Bucur, M. and Schirmacher, V. 2006. Tumor selective replication of Newcastle disease virus: Association with defects of tumor cells in antiviral defence. *International Journal of Cancer*, 119(2): 328-338.

Galbiati, F., Engelman, J.A., Volonte, D., Zhang, X.L., Minetti, C., Li, M., Hou jr, H., Kneitz, B., Edelman, W. & Lisanti, M.P. (2001) Caveolin-3 null mice show a loss of caveolae, changes in the microdomain distribution of the dystrophin-glycoprotein complex, and T-tubule abnormalities. *Journal of Biological Chemistry*, 276: 21425-21433.

Gallili, G. E., and Ben-Nathan, D. 1998. Newcastle disease vaccines. *Biotechnology Advances* in *Elsevier Science,* 16: 343-366.

Garcia-Sastre, A., Gabezas, J. A., and Villar, E. 1989. Protein of Newcastle disease virus envelope: interaction between the outer hemagglutinin-neuraminidase glycoprotein and the inner non-glycosylated matrix protein. *Biochimica et Biophysica Acta,* 999, 171-175.

Ghigo E, Kartenbeck J, Lien P, Pelkmans L, Capo C, et al. 2008. Ameobal Pathogen Mimivirus infects macrophages through phagocytosis. *PLoS Pathogens*, 4: e1000087. doi:10.1371/journal.ppat.1000087.

Harder, T. and Simons, K. 1997. Caveolae, DIGs, and the dynamics of sphingolipid-cholestrol microdomains. *Current Opinion in Cell Biology*, 9: 534-542.

Horvath, J.C., Horak, H., Sinkovics, J.G., Pritchard, M., Pendleton, S. and Horvath, E. 2000. Cancer vaccines with emphasis on the viral oncolysate melanoma vaccine. *Acta Microbiologica et Immunologica Hungarica*, 46: 1-20.

Huang, Q.Y, Li, X.F. and Liu, S.P. 2012. E-cadherin and caveolin-1 alterations in the heart of rats having undergone chlorpromazine-induced toxicity. *Molecular Medicine Reports*, 5(3): 705-709.

Ivanov, A.I. 2008. Pharmacological inhibition of endocytic pathways: Is it specific enough to be useful. From: Methods in Molecular Biology, Vol. 440, Exocytosis and Endocytosis, ed. A.I. Ivanov, pp. 15-33. Humana Press, Totowa, NJ.

Karp, G. 2005. Cell and molecular biology: concepts and experiments, 4th edition. *John Wiley & Sons, Inc.*

Kartenbeck, J., Stukenbrok, H. and Helenius, A. 1989. Endocytosis of simian virus 40 into the endoplasmic reticulum. *Journal of Cell Biology,* 109: 2721-2729.

Kim, J.H., Jung, S.Y., Lee, Y.J., Song, K.J., Kwon, D., Park, S., Im, K.I. and Shin, H.J. 2008. Effect of therapeutic chemical agents in vitro and on experimental meningoencephalitis due to Naegleria fowleri. *Antimicrobial Agents and Chemotherapy*, 52(11): 4010-4016.

Kolakofsky, D., Boy De La Tour, E., and Delivs, H. 1974. Molecular weight determine at Sendai and NDV RNA. *Journal Virology,* 13: 261-268.

Lai, M.C. and Ibrahim, A.L. 1987. Velogenic Viscerotropic Newcastle Disease Virus. In: Proceedings Newcastle Disease Workshop., 16-20 March 1987., UPM, Serdang.

Lamaze, C., Dejeancourt, A., Baba, T., Lo, C.G., Benmerah, A. and Dautry-Varsat, A. 2001. Interleukin 2 receptors and detergen-resistant membrane domains define a clathrin-independent endocytic pathway. *Molecular Cell,* 7: 661-671.

Lamb, R.A. and Kolakofsky, D. 1996. *Paramyxoviridae*: The viruses and their replication. In B.N. Fields, D.M. Knipe & P.M. Howley (Eds.), *Fields virology,* 3rd edition, Vol. 1: 1177-1203. Philadelphia, PA: Lippincott-Raven Publishers.

Lecot, S., Belouzard, S., Dubuisson, J. and Rouille, Y. 2005. Bovine viral diarrhea virus entry is dependent on clathrin-mediated endocytosis. *Journal of Virology*, 79:10826–10829

Lee, H.P., Gourley, L., Duffy, S.W., Esteve, J., Lee, J., and Day, N.E. 1991. Dietary effects on breast-cancer risk in Singapore. *Lancet*, 337: 1197-1200.

Liu, Y., Peterson, D.A., Kimura, H. and Schubert, D. 1997. Mechanism of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction. *Journal of Neurochemistry*, 69(2): 581-593.

Marsh, M. and McMohan, H.T. 1999. The structural era of endocytosis. *Science* 285: 215-220.

Marsh, M. and Helenius, A. 2006. Virus entry: open sesame. *Cell*, 124(4): 729-740.

Martin, J.J., Holguera, J., Sanchez-Felipe, L., Villar, E. and Munoz-Barroso, I. 2012. Cholestrol dependence of Newcastle disease virus entry. *Biochimica et Biophysica Acta – Membrane*; 1818(3): 753-761.

Mascotti, K., McCullough, J., and Burger, S.R., 2000. HPC viability measurement: trypan blue versus acridine orange and propidium iodide. *Transfusion* 40: 693-696.

Mayo, M. A. 2002. A summary of taxonomic changes recently approved by ICTV. *Arch. Of Virology*, 147: 1655-1656.

McKimm-Berschkin, J.L. 2004. A simplified plaque assay for respiratory syncytial virus - direct visualization of plaques without immunostaining. *Journal of Virological Methods*, 120(1): 113-117.

Mercier, I., Jasmin, J.F., Pavlides, S., Minetti, C., Flomenberg, N., Pestell, R.G., Frank, P.G., Sotgia, F., and Lisanti, M.P. 2009. Clinical and translational implications of the caveolin gene family: lessons from mouse models and human genetic disorders. *Laboratory Investigation*, 89, 614-623.

Millar, N.S., and Emmerson, P.T. 1988. Molecular cloning and nucleotide sequencing of NDV. In D.J. Alexander (ed.) *ND Kluwer Academic Publishers, Boston*, pp 79-97.

Minshall, R.D., Sessa, W.C., Stan, R.V., Anderson, R.G. and Malik A.B. 2003. Caveolin regulation of endothelial function. *American Journal of Physiology. Lung cellular and molecular physiology*, 285(6): L1179-1183.

Norkin, L.C. and Kuksin, D. 2005. The caveolae-mediated SV40 entry pathway bypasses the golgi complex *en route* to the endoplasmic reticulum. *Virology Journal* 2: 38.

Osborne, C., and Brooks, S.A. 2006. SDS-PAGE and western blotting to detect proteins and glycoproteins of interest in breast cancer research. Breast Cancer Research Protocols, Methods in Molecular Medicine, 120: pp 217-229. Humana Press Inc. Totowa, NJ.

Parton, R.G. and Richards, A.A. 2003. Lipid rafts and caveolae as portals for endocytosis: new insights and common mechanisms. *Traffic*, 14(11): 724-738

Parton, R.G. and del Pozo, M.A. 2013. Caveolae as plasma membrane sensors, protectors and organizers. *Nature*, 14: 98-112.

Payment, P., and Trudel, M. 1993. Isolation and identification of viruses. *In*: Method and techniques of virology. Payment, P. and Trudel (ed.). Marcell Dekker Inc., New York.

Peeters, B.P., De Leeuw, O.S., Koch, G., and Gielkens, A.L. 1999. Rescue of Newcastle disease virus from cloned cDNA: evidence that cleavability of the fusion protein is a major determinant for virulence. *Journal of Virology* 73: 5001-5009.

Pelkmans, L., Puntener, D., and Helenius, A. 2002. Local actin polymerization and dymin recruitment in SV40-induced internalization of caveolae. *Science* 296: 535-539.

Peterson, G. 1995. Evaluation of the biochemical targets of genistein in tumor cells. *The Journal of Nutrition*, 125: 784S-789S

Pho, M.T., Ashok, A. and Atwood, W.J. 2000. JC virus enters human glial cells by clathrin dependent receptor mediated endocytosis. *Journal of Virology*, 74: 2288–2292

Pike, L.J. 2006. Rafts defined: a report on the Keystone Symposium on Lipid Rafts and Cell Function. *Journal of Lipid Research*, 47(7): 1597-1598.

Pike, L.J. 2008. The challenge of lipid rafts. *Journal of Lipid Research*, 50: 1-17.

Querbes, W., Benmerah, A., Tosoni, D., Di Fiore, P.P., and Atwood, W.J. 2004. A JC Virus-induced signal is required for infection of glial cells by a clathrin- and eps15- dependent pathway. *Journal of Virology* 78, 1, 250-256.

Rajendran, L. and Simons, K. 2005. Lipid rafts and membrane dynamics. *Journal of Cell Science* 118: 1099-1102.

Reichard, K.W., Lorence, R.M., Cascino, C.J., Peeples, M.E., Walter, R.J., Fernando, M.B., Reyes, H.M. and Grenger, J.A. 1992. Newcastle disease virus selectively kills human tumour cells. *Journal of Surgical Research*, 52, 448-453.

Ring, C.J.A. 2002. Cytolytic viruses as potential anti-cancer agents. *Journal of General Virology*, 83: 491-501

Rott, R. and Klenk, H.D. 1988. Molecular basic of infectivity and pathogenicity of Newcastle disease virus. In D.J. Alexander (ed.) *ND Kluwer Academic Publisher, Boston*, pp 98-112.

Rust, M. J., Lakadamyali, M., Zhang F., and Zhuang, X. 2004. Assembly of endocytic machinery around individual influenza viruses during viral entry. *Nature Structural and Molecular Biology*, 11: 567-573.

Samaj, J., Baluska, F., Voigt, B., Schlicht, M., Vilkmann, D. and Menzel, D. 2004. Endocytosis, actin cytoskeleton, and signaling. *Plant Physiology*, 135:11150-1161.

Samson, A.C.R. 1988. Virus structure. In: D.J Alexander (ed) Newcastle disease. *Kluwer Academic Publishers*, pp 23-44.

San Roman, K., Villar, E., and Munoz-Barroso, I. 1999. Acidic pH enhancement of the fusion of Newcastle disease virus with cultured cells. *Virology* 260: 329-341.

Schirrmacher, V., Bai, L., Umansky, V., Yu, L., Xing, Y. and Qian, Z. 2000. Newcastle disease virus activates macrophages for antitumor activity. *International Journal of Oncology*, 16: 363-373.

Sieczkarski, S.B., and Whittaker, G.R. 2002. Dissecting virus entry via endocytosis. *Journal of General Virology*, 83: 1535-1545.

Sieczkarski, S.B., and Whittaker, G.R. 2005. Viral entry. *Current Topics in Microbiology and Immunology*, 285: 1-23.

Stan, R. V. 2005. Structure of caveolae. *Biochimica et Biophysica Acta* 1746: 334-348.

Stanbridge, E.J. 1971. Mycoplasmas and cell cultures. *Bacteriological Reviews*, 35: 206-226.

Stone-Hulslander, J. and Morisson, T.G. 1997. Detection of an interaction between the HN and F proteins in Newcastle disease virus-infected cells. *Journal of Virology*, 71: 6287-6295.

Swanson, J.A, and Watts, C. 1995. Macropinocytosis. *Trends Cell Biology* 5: 424–428.

Takahashi, T. and Suzuki, T. 2009. Role of membrane rafts in viral infection. *The Open Dermatology Journal*, 3, 178-194.

Tan, W.S., Lau, C.H., Ng, B.K., Ibrahim, A.L., and Yusoff, K. 1995. Nucleotide sequence of the haemagglutination-neuraminidase (HN) gene of a Malaysian heat resistant viscerotropic-velogenic Newcastle disease virus. *DNA Sequence*, 6: 47-50.

Tortora, G.J., Funke, B.R. and Case, C.L. 2007. Observing microorganisms through a microscope. Microbiology; an introduction, 9th edn., pp 61-64. *Pearson Education, Inc.*

Tsukiyama, K., Yoshikawa, Y., and Yamanorchi, K. 1988. Fusion glycoprotein (F) of Rinderpest virus; entire nucleotide sequence of F mRNA, and several futures of the protein. *Virology* 164: 523-530.

Umino, Y., Kohana, T., Kohase, M., Sugivra, A., Klenky, H.D., and Rott, R. 1984. Biological function of monospecific antibodies envelope glycoproteins of NDV. *Archives of Virology* 81: 53-65.

Van der Bliek, A.M., Redelmeier, T.E., Damke, H., Tisdale, E.J., Meyerwitz, E.M. and Schmid, S.L. 1993. Mutation in human dynamin block an intermediate stage in coated vesicle formation. *Journal of Cell Biology*, 122: 553-563.

Vela, E.M., Bowick, G.C., Herzog, N.K. and Aronson, J.F. 2008. Genistein treatment of cells inhibits Arenavirus infection. *Antiviral Research*, 77(2): 153-156.

Villar, E. and Barroso, I.M. 2006. Role of sialic acid-containing molecules in paramyxovirus entry into the host cells: A minireview. *Glycoconjugate Journal*, 23(1-2): 5-17.

William, T.M. and Lisanti, M.P. 2004. Protein family review: the caveolin proteins. *Genome Biology*. 5: 214.1-214.8.

Yusoff, K., and Tan, W.S. 2001. Newcastle disease virus: macromolecules and oppurtunities. *Avian Pathology*, 30: 439-455.

Zaitsev, V., von Itzstein, M., Groves, D., Kiefel, M., Takimoto, T., Portner, A. and Taylor, G. 2004. Second sialic acid binding site in Newcastle disease virus hemagglutinin-neuraminidase: implications for fusion. *Journal of Virology*, 78: 3733–3741.

Zinchuk, V., Zinchuk, O. and Okada, T. 2007. Quantitative colocalization analysis of multicolor confocal immunofluorescence microscopy images: Pushing pixels to explore biological phenomena. *Acta Histochemica et Cytochemica*, 40(4): 101-111.