

# Modulación de la infección por el ISAV mediante inhibidores de endocitosis mediada por clatrina y macropinocitosis

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- Al Soraj, M., He, L., Peynshaert, K., Cousaert, J., Vercauteren, D., Braeckmans, K., et al. 2012. siRNA and pharmacological inhibition of endocytic pathways to characterize the differential role of macropinocytosis and the actin cytoskeleton on cellular uptake of dextran and cationic cell penetrating peptides octaarginine (R8) and HIV-Tat. *J. Control Release*, 161: 132-141.
- Aspehaug, V., Falk, K., Krossøy, B., Thevarajan, J., Sanders, L., Moore, L., et al. 2004. Infectious salmon anemia virus (ISAV) genomic segment 3 encodes the viral nucleoprotein (NP), an RNA-binding protein with two monopartite nuclear localization signals (NLS). *Virus Res.*, 106: 51-60.
- Bauherr, S., Larsberg, F., Petrich, A., Sperber, H., Klose-Grzelka, V., Luckner, M., et al. 2020. Macropinocytosis and Clathrin-Dependent Endocytosis Play Pivotal Roles for the Infectious Entry of Puumala Virus. *J. Virol.*, 94 (14). Bloomfield, G. & Kay, R. 2016. Uses and abuses of macropinocytosis. *J. Cell Sci.*, 129: 2697-2705.
- Cardenas, M., Michelson, S., Perez, D., Montoya, M., Toledo, J., Vasquez- Martinez, Y., et al. 2022. Infectious Salmon Anemia Virus Infectivity Is Determined by Multiple Segments with an Important Contribution from Segment 5. *Viruses*. 14(3).
- Commissio, C., Flinn, R. & Bar-Sagi, D. 2014. Determining the macropinocytic index of cells through a quantitative image-based assay. *Nat. Protoc.*, 9: 182-192.
- Cottet, L., Rivas-Aravena, A., Cortez-San Martin, M., Sandino, A. & Spencer, E. 2011. Infectious salmon anemia virus—genetics and pathogenesis. *Virus Res.*, 155:10-19.
- Dannevig, B., Falk, K. & Namork, E. 1995. Isolation of the causal virus of infectious salmon anaemia (ISA) in a long-term cell line from Atlantic salmon head kidney. *J. Gen. Virol.* 76: 1353-1359.
- Delpeut, S., Sisson, G., Black, K. & Richardson, C. 2017. Measles Virus Enters Breast and Colon Cancer Cell Lines through a PVRL4-Mediated Macropinocytosis Pathway. *J. Virol.*, 91 (10). de Vries, E., Tscherne, D., Wienholts, M., Cobos-Jiménez, V., Scholte, F., García-Sastre, A., et al. 2011. Dissection of the Influenza A Virus Endocytic Routes Reveals Macropinocytosis as an Alternative Entry Pathway. *PLOS Pathogens*, 27: e1001329.
- Doherty, G. & McMahon, H. 2009. Mechanisms of endocytosis. *Annu Rev Biochem.* 78: 857-902.
- Eierhoff, T., Hrinicins, E., Rescher, U., Ludwig, S. & Ehrhardt, C. 2010. The epidermal growth factor receptor (EGFR) promotes uptake of influenza A viruses (IAV) into host cells. *PLoS Pathog.*, 6: e1001099. Eliassen, T., Froystad, M., Dannevig, B., Jankowska, M., Brech, A., Falk, K., et al. 2000. Initial events in infectious salmon anemia virus infection: evidence for the requirement of a low-pH step. *J. Virol.*, 74: 218-227.
- Fosse, J., Andresen, A., Ploss, F., Weli, S., Heffernan, I., Sapkota S, et al. 2023. The infectious salmon anemia virus esterase prunes erythrocyte surfaces in infected Atlantic salmon and exposes terminal sialic acids to lectin recognition. *Front. Immunol.*, 14: 1158077.
- Fosse, J., Aamelfot, M., Sonstevold, T., Weli, S., Vendramin, N., Petersen, P., et al. 2022. Salmon Erythrocytes Sequester Active Virus Particles in Infectious Salmon Anaemia. *Viruses*, 14(2).
- Godoy, M., Suarez, R., Lazo, E., Llegues, K., Kibenge, M., Wang, Y. & Kibenge, F. 2014. Genetic analysis and comparative virulence of infectious salmon anemia virus (ISAV) types HPR7a and HPR7b from recent field outbreaks in Chile. *Virol. J.*, 11: 204.
- Gold, S., Monaghan, P., Mertens, P. & Jackson, T. 2010. A clathrin independent macropinocytosis-like entry mechanism used by bluetongue virus-1 during infection of BHK cells. *PLoS One*, 5: e11360.
- Hellebo, A., Vilas, U., Falk, K. & Vlasak, R. 2004. Infectious salmon anemia virus specifically binds to and hydrolyzes 4-O-acetylated sialic acids. *J. Virol.*, 78: 3055-3062.
- Haspot, F., Lavault, A., Sinzger, C., Laib, Sampaio, K., Stierhof, Y., Pilet, P., et al. 2012. Human cytomegalovirus entry into dendritic cells occurs via a macropinocytosis-like pathway in a pH-independent and cholesterol-dependent manner. *PLoS One*, 7: e34795.
- Jones, A. 2007. Macropinocytosis: searching for an endocytic identity and role in the uptake of cell penetrating peptides. *J. Cell Mol. Med.*, 11: 670-684.
- Kerr, M. & Teasdale, R. 2009. Defining macropinocytosis. *Traffic*. 10: 364-371.
- Koivusalo, M., Welch, C., Hayashi, H., Scott, C., Kim, M., Alexander, T., et al. 2010. Amiloride inhibits macropinocytosis by lowering submembranous pH and preventing Rac1 and Cdc42 signaling. *J. Cell Biol.*, 188: 547-563.
- Kumar, C., Dey, D., Ghosh, S. & Banerjee, M. 2018. Breach: Host Membrane Penetration and Entry by Nonenveloped Viruses. *Trends. Microbiol.*, 26: 525-537.
- Levican, J., Miranda-Cardenas, C., Soto-Rifo, R., Aguayo, F., Gaggero, A. & Leon, O. 2017. Infectious pancreatic necrosis virus enters CHSE-214 cells via macropinocytosis. *Sci. Rep.*, 3068.
- Levican-Asenjo, J., Soto-Rifo, R., Aguayo, F., Gaggero, A. & Leon, O. 2019. Salmon cells SHK-1 internalize infectious pancreatic necrosis virus by macropinocytosis. *J. Fish Dis.*, 42: 1035-1046.

- Lim, J. & Gleeson, P. 2011. Macropinocytosis: an endocytic pathway for internalising large gulps. *Immunol. Cell Biol.*, 89: 836-843.
- Lorizate, M. & Krausslich, H. 2011. Role of lipids in virus replication. *Cold Spring Harb Perspect Biol.*, 3: a004820.
- Mardones, F., Martinez-Lopez, B., Valdes-Donoso, P., Carpenter, T. & Perez, A. 2014. The role of fish movements and the spread of infectious salmon anemia virus (ISAV) in Chile, 2007-2009. *Prev. Vet. Med.*, 114: 37-46.
- Marsh, M. & Helenius, A. 2006. Virus entry: open sesame. *Cell*, 124: 729-740.
- Mercer, J., Schelhaas, M. & Helenius, A. Virus entry by endocytosis. 2010. *Annu Rev Biochem.*, 79: 803-833.
- Meier, O., Boucke, K., Hammer, S., Keller, S., Stidwill, R., Hemmi S, et al. 2002. Adenovirus triggers macropinocytosis and endosomal leakage together with its clathrin-mediated uptake. *J. Cell Biol.*, 158: 1119-1131.
- Mercer, J. & Helenius, A. 2009. Virus entry by macropinocytosis. *Nat. Cell Biol.*, 211: 510-520.
- Mikalsen, A., Sindre, H., Mjaaland, S. & Rimstad, E. 2005. Expression, antigenicity and studies on cell receptor binding of the hemagglutinin of infectious salmon anemia virus. *Arch Virol.*, 150: 1621-1637.
- Ritchie, R., McDonald, J., Glebe, B., Young-Lai, W., Johnsen, E. & Gagne, N. 2009. Comparative virulence of Infectious salmon anaemia virus isolates in Atlantic salmon, *Salmo salar* L. *J. Fish. Dis.*, 32: 157-171.
- Rivas-Aravena, A., Vallejos-Vidal, E., Cortez-San Martin, M., Reyes-Lopez, F., Tello, M., Mora P, y col. 2011. Inhibitory effect of a nucleotide analog on infectious salmon anemia virus infection. *J Virol.*, 85: 8037-8045.
- Rossmann, J., Leser, G. & Lamb, R. 2012. Filamentous influenza virus enters cells via macropinocytosis. *J. Virol.*, 86: 10950-10960.
- Toennessen, R., Lauscher & A. Rimstad, E. 2009. Comparative aspects of infectious salmon anemia virus, an orthomyxovirus of fish, to influenza viruses. *Indian J. Microbiol.*, 49: 308-314.
- Ramirez, R. & Marshall, S. 2018. Identification and isolation of infective filamentous particles in Infectious Salmon Anemia Virus (ISAV). *Microb Pathog.*, 17: 219-224.
- Saeed, M., Kolokoltsov, A., Albrecht, T. & Davey, R. 2010. Cellular entry of ebola virus involves uptake by a macropinocytosis-like mechanism and subsequent trafficking through early and late endosomes. *PLoS Pathog.*, 6: e1001110.
- Sanchez, E., Quintas, A., Perez-Nunez, D., Nogal, M., Barroso, S., Carrascosa, A., et al. 2012. African swine fever virus uses macropinocytosis to enter host cells. *PLoS Pathog.*, 8: e1002754.
- Schink, K., Tan, K., Spangenberg, H., Martorana, D., Sneeggen, M., Stevenin, V., et al. 2021. The phosphoinositide coincidence detector Phafin2 promotes macropinocytosis by coordinating actin organisation at forming macropinosomes. *Nat. Commun.*, 12: 6577.
- Sieben, C., Sezgin, E., Eggeling, C. & Manley, S. 2020. Influenza A viruses use multivalent sialic acid clusters for cell binding and receptor activation. *PLoS Pathog.*, 16: e1008656.
- Shevchuk, A., Hobson, P., Lab, M., Klenerman, D., Krauzewicz, N. & Korchev, Y. 2008. Endocytic pathways: combined scanning ion conductance and surface confocal microscopy study. *Pflugers Arch.*, 456: 227-235.
- Sun, E., Liu, A., Zhang, Z., Liu, S., Tian, Z. & Pang, D. 2017. Real-Time Dissection of Distinct Dynamin-Dependent Endocytic Routes of Influenza A Virus by Quantum Dot-Based Single-Virus Tracking. *ACS Nano*. 11: 4395-4406.
- Swanson, J. & Watts, C. 1995. Macropinocytosis. *Trends Cell Biol.*, 5: 424-428.
- Tkachenko, E., Lutgens, E., Stan, R. & Simons, M. 2004. Fibroblast growth factor 2 endocytosis in endothelial cells proceed via syndecan-4-dependent activation of Rac1 and a Cdc42-dependent macropinocytic pathway. *J. Cell Sci.*, 117: 3189-3199.
- Vallejos, E., Sandino, A., Rivas, A., Spencer, E. & Modak, B. 2012. Antiviral activity in vitro and in vivo of natural flavonoids isolated from *Heliotropium sinuatum* against infectious salmon anemia virus (ISAV). *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*. 11: 377-384.
- Wang, S., Huang, X., Huang, Y., Hao, X., Xu, H., Cai, M, et al. 2014. Entry of a novel marine DNA virus, Singapore grouper iridovirus, into host cells occurs via clathrin-mediated endocytosis and macropinocytosis in a pH-dependent manner. *J Virol.*, 88: 13047-1363.
- West, M., Bretscher, M. & Watts, C. 1989. Distinct endocytotic pathways in epidermal growth factor-stimulated human carcinoma A431 cells. *J. Cell Biol.*, 109:2731-2739.
- Workenhe, S., Wadowska, D., Wright, G., Kibenge, M. & Kibenge, F. 2007. Demonstration of infectious salmon anaemia virus (ISAV) endocytosis in erythrocytes of Atlantic salmon. *Virol. J.*, 4: 13.