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NHE1 (Na⁺/H⁺ exchanger 1) promotes invadopodia ECM degradation and invasion through the spatially restricted acidification of the peri-invadopodial space
Busco G¹, Cardone R.A.¹, Belluzzi A.¹,², Greco M.R.¹, Antelmi E.³, Casavola V.¹, Paradiso A.³, Reshkin S.J.¹*¹
¹Dept. General and Environmental Physiology, University of Bari, Via Amendola 165/A, 70126, Bari, Italy; reshkin; ²Clinical Experimental Oncology Laboratory, National Cancer Institute Giovanni Paolo II, Via Hahnemann 10, 70126, Bari, Italy.
*Contact author: reshkin@biologia.uniba.it

Degradation of the extracellular matrix (ECM) is a critical process of tumor cell invasion and requires membrane and released proteases localized at membrane structures called invadopodia. While extracellular acidification is important in driving tumor invasion, the structure/function mechanisms driving it are still unknown. Invadopodia are very similar in structure and function to osteoclast podosomes responsible for bone degradation. Extracellular acidification is central to podosome action and, by analogy, could also be for invadopodial function. Here, we show that NHE1 and NHE1-dependent extracellular acidification are localized at invadopodia and are necessary for tumor cell matrix-degrading activity. Experiments were conducted in metastatic breast cancer cells seeded onto matrigel in which quenched BSA- or collagen-FITC was mixed and invadopodia activity evaluated microscopically. Focal proteolysis produces fluorescence which is used to quantitatively measure proteolytic activity, co-localization analysis of NHE1 expression and extracellular pH. Immunofluorescence showed that invadopodia-dependent focal ECM degradation is tightly associated with NHE1 expression and that NHE1 often co-localized with cortactin. Areas of focal ECM digestion had more acidic pH values compared to the edge of the cells where only pericellular digestion had occurred and the acidification was blocked by the specific NHE1 inhibitor, cariporide (HOE642). Stimulation with EGF increased both ECM degradation and NHE1-dependent proton secretion. Exposure of tumor cells to low medium pH, low serum or hypoxia stimulated invadopodia-dependent ECM proteolysis. Exposure of tumor cells to low medium pH increased both NHE1 activity and invadopodial-dependent ECM proteolysis with a decrease in invadopodial distribution, length and association with NHE1. Manipulation of the NHE1 expression level or activity by RNA interference, transport-deficient mutation or the specific inhibitor cariporide confirmed that NHE1 expression and activity are required for invadopodia-mediated ECM degradation. We conclude that NHE1 and its associated extracellular acidification are localized to cancer cell invadopodia and are necessary for invadopodial ECM digestion.

References
Yasumasa Kato*
Department of Biochemistry and Molecular Biology, Kanagawa Dental College, Yokosuka, Japan
*Contact author: yasumasa@kdcnet.ac.jp

The extracellular pH (pHe) of tumor tissues has been well known to be often acidic. Although the acidification is believed to be mainly due to acidic metabolites, e.g., lactate, caused by anaerobic glycolysis, recent studies have investigated that CO₂ from pentose phosphate pathway is also a major source of acidity. In earlier study, we reported that acidic pH, up-regulated production of matrix metallo-proteinase-9 (MMP-9) / gelatinase B that plays an important role of type IV collagen degradation in tumor metastasis. Thereafter, other pro-metastatic factors, such as vascular endothelial cell growth factor (VEGF) and interleukin-8 (IL-8), have been reported to be acidic pH-regulated gene by several research groups. We further explored the intracellular signaling pathway for acidic pH signaling to induce MMP-9 expression as the target gene. Acidic pH significantly activated phospholipase D (PLD), but not phosphatidylinositol-specific phospholipase C. Proximal promoter assay for MMP-9 revealed that NFκB binding site was a major responsible element for the acidic pH L. PLD activation conducted to mitogen-activated kinases followed by NFκB activation. Furthermore, we found that Ca²⁺-influx triggered PLD activation and that acidic sphingomyelinase and protein kinase Cζ were associated with NFκB activation. In addition, RhoA, but not Rac1 andcdc42, was also involved in acidic pH signaling. Because hypoxia inducible factor 1α was induced by acidic pH, but not by hypoxia, cellular activity of tumor cells was regulated by acidic pH and hypoxia at different stage of extracellular microenvironment in the tumor tissue.