ALK-mediated Na\(^+\)/H\(^+\) exchanger-dependent intracellular alkalization: does it matter for oncogenesis?

In this study we investigated the relevance of the oncogenic protein NPM-ALK in regulating cellular pH (pH) through the modulation of Na\(^+\)/H\(^+\) exchanger 1 (NHE1)-activity and the consequences of pH pharmacological manipulation in cells expressing NPM-ALK.

We have recently reviewed the biological relevance of ALK and related fusion proteins in oncogenesis. A recent study has pointed out that the Na\(^+\)/H\(^+\) exchanger (NHE1)-dependent alkaline pH is an important event in the malignant-transformation associated phenotype. NHE1 is a commonly expressed plasma membrane protein that regulates pH through an exchange of extracellular Na\(^+\) for intracellular H\(^+\). We have recently shown that troglitazone (TRO) induces prolonged cellular acidosis by inhibiting NHE1 activity and glutamine (GLU) oxidation via the transamination pathway forming alanine (ALA), shifting glutamate (Glu) into ammoniogenesis via oxidative deamination by alanine aminotransferase (ALT), and glutamate dehydrogenase (GDH). Hence tumor cells express negligible or low levels of GDH. Their dependency upon GLU for rapid growth and proliferation is entirely dependent upon the transamination-pathway. To test whether TRO would be effective in arresting proliferation of ALK

![Graph A](image1.png)

**Figure 1. A.** Left side. [\(^{3}H\)]thymidine labeling in ALK-ALCL-derived cells L82. Cells were incubated in fresh media plus 1 \(\mu\)Ci/mL [\(^{3}H\)]thymidine for 18 hours treated with 40 \(\mu\)M TRO or DMSO (control). Results are shown as count per minute (cpm)/mg. **Right side.** Caspase 3/7 activity determined as described in the text and represented as RFLUs. **B.** NIH3T3 cell proliferation is increased by NPM-ALK exogenous expression. Cells after stable transfection and assessed for expression of NPM-ALK (not shown) were plated in 12 well-dish. Proliferation was followed for 72 hours by counting cells by trypan blue exclusion. Conditioned media was collected at all time points and used for metabolic analysis. An inset panel shows the presence of long pseudopodia in ALK-NIH3T3 cells. **C.** NHE1 exchanger activity is altered by NPM-ALK expression. Cells were plated on specially designed coverslips for exchanger activity measurements. Cells were loaded with pH sensitive dye (BCECF) in Krebs Hensleit Hepes media (KHH). After establishment of baseline pH, cells were pulsed with NH\(_4\)Cl for 4 mins followed by KHH-recovery time: SR: slow recovery; RR: rapid recovery after acid load. From Dr. S. Morris, St. Jude Children's Research Hospital, Memphis, TN, USA) as previously described. A striking change in cell morphology was observed in ALK

![Graph B](image2.png)

![Graph C](image3.png)

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the recovery phase. Baseline pH in ALK-NIH3T3 cells of 7.45 confirmed that as expected these cells were more alkaline than the control at 7.34 (Figure 1C). Our data support a pH-mediated oncogenic activity of NPM-ALK protein through the regulation of the NHE1 activity that favors alkaline conditions by increasing the extrusion of cellular H⁺. The increased NPM-ALK mediated alkaline conditions favor growth and proliferation that are reversed by TRO-induced cellular acidosis in ALK-ALCL-derived L82 cells. Our results support the study of drugs that induce cellular acidosis either by interfering with acid extrusion or acid production or, ultimately, by a combination of both in tumors induced by oncogenic alkaline fusion proteins.*

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