ALK-mediated Na^+/H^+ exchanger-dependent intracellular alkalinization: does it matter for oncogenesis?

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

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We have recently reviewed the biological relevance of ALK and related fusion proteins in oncogenesis.[1] A recent study has pointed out that the Na^+/H^+ exchanger (NHE)1-dependent alkaline pH_i is an important event in the malignant-transformation associated phenotype.[2] NHE1 is a commonly expressed plasma membrane protein that regulates pHi through an exchange of extracellular Na^+ for intracellular H^+.[3] 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).[4,5] 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.[5] To test whether TRO would be effective in arresting proliferation of ALK^+/ALCL (anaplastic large cell lymphoma)-derived L82 cells by inducing cellular acidosis and inhibiting GLU oxidation, cells were incubated for 18 h in the presence of 40 μM TRO or vehicle (DMSO), and GLU metabolism and DNA synthesis were determined as previously described.[4,5] TRO-treated cells utilized far less GLU (883±789 vs 3190±270 nmoL/mg protein, $p<0.05$) as a result of marked inhibition ($p<0.01$) of the pH-dependent transamination reaction (ALA production decreasing from 1293±278 to 218±54 nmol/mg protein). On the other hand, ammonium production equalled GLU utilization consistent with high glutaminase activity of tumor cells, but it failed to increase with TRO (2038±537 vs 3340±637 nmoL/mg for control) reflecting the low activity of GDH in tumor cells. We have previously shown that TRO-induced cellular acidosis (peroxisome proliferator-activated receptor γ (PPARγ)-independent) is associated with reduced DNA synthesis in breast cancer-derived cells.[4] As shown in Figure 1A, left side, ^{3}H-thymidine incorporation was decreased in TRO-L82 cells [3252±482 vs L82-control cells 5745±353 ($p<0.005$)]. We also measured the caspase 3/7 activity since we have recently shown that TRO-induced cellular acidosis is associated with increased mitochondrial permeability.[6] L82 cells incubated with 40 μM TRO showed a significant increase of caspase activity measured with CellProbe™ HT Caspase 3/7 Whole Cell Assay, Beckman Coulter, as compared to control at 18 hours (Figure 1A, right side, TRO-L82 cells 6306±134 vs L82-control cells 2063±70, $p<0.001$). Given the relevance of the consequences of pharmacological manipulation of pH_i in ALK^+ cells L82, we assessed whether NPM-ALK played any role in regulating the pH_i. For this purpose, we stably transfected NIH3T3 fibroblasts with a pSRoMSVtkneo vector expressing NPM-ALK (*a gift from Dr. S. Morris, St. Jude Children's Research Hospital, Memphis, TN, USA*) as previously described.[7] A striking change in cell morphology was observed in ALK^+-NIH3T3 cells that transformed from a single monolayer (controls) to elongated cells with long pseudopodia (Figure 1B, inset). ALK^+-NIH3T3 cells proliferated at a faster rate than parental cells, with a 1.5 fold increase in cell number over a period of 72 h (Figure 1B, $p<0.02$). Ratio of ammonia (NH4) to alanine (ALA) was used as a surrogate marker of pH_i as previously described.[5] Analysis of the conditioned media from control and ALK^+-NIH3T3 cells showed a 4-fold decrease in the ratio of ammonia to ALA (NH_4/ALA 16.3 control vs 4.0 for transfectants) consistent with an alkaline pH_i and increased proliferation as previously shown.[2,5] Subsequently, NHE1 activity was determined as the rate of pH_i recovery after an ammonia-chloride (NH_4Cl) load and assayed using (2,7)-biscarboxyethyl-5(6)-carboxyfluorescein (BCECF) as previously described.[4,5] As shown in Figure 1C, the untransformed NIH3T3 cells (left side) have modest NHE1 activity ($\Delta pH/\Delta_t$) compared to the ALK^+-NIH3T3 cells (right side) in response to the NH_4Cl load as represented by the difference in

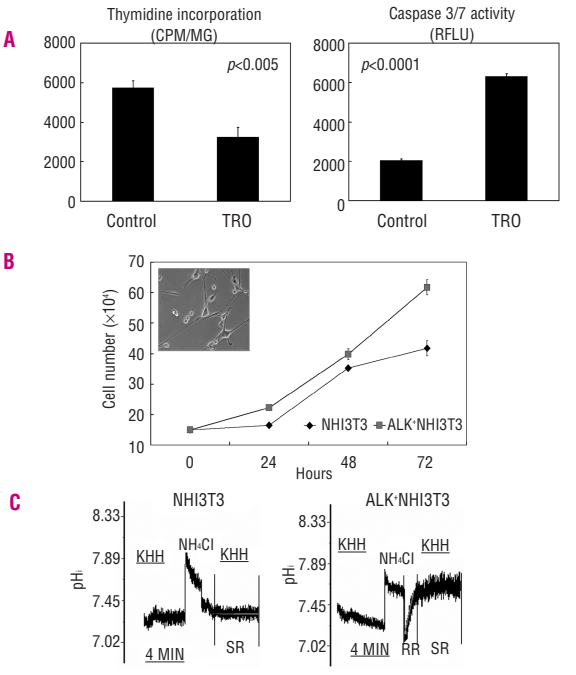

Figure 1. A. ***Left side.*** **[^{3}H]thymidine labeling in ALK^+ALCL-derived cells L82. Cells were incubated in fresh media plus 1 μCi/mL [^{3}H]thymidine for 18 hours treated with 40 μM TRO or DMSO (control). Results are shown as count per minute (cpm)/mg.** ***Right side.*** **Caspase 3/7 activity 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_4Cl for 4 mins followed by KHH-recovery time: SR: slow recovery; RR: rapid recovery after acid load.**

the recovery phase. Baseline pH_i 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.[8]

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