Increased migration potential of lung cancer cells in co-culture conditions with lung fibroblasts is metabolism dependent

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Introduction

The metabolic interactions between cancer cells and the adjacent stroma fibroblasts and endothelial cells, remain an open area of research. In previous studies we have shown that in most tumors, cancer cells retain a strong expression of enzymes and proteins involved in glucose absorption and glycolysis, while the adjacent fibroblasts have an aerobic metabolism profile.

Aim

In the current study, we attempted to investigate the paracrine interactions between lung fibroblasts MRC5 and lung cancer H1299 and A549 cells, growing within the same culture medium, by studying effects on cancer cell migration with the IBIDI Single Culture-Insert migration assay.

Materials and methods

Silencing (siRNAs)

Human lung cancer cell lines A549 and H1299 and human normal fibroblast MRC5 were cultured and maintained using standard procedures. siRNAs were custom synthesized (GenePharma Co, Shanghai, China), pooled and used at 100 nM to transfect cancer cells with PDK and LDHA(V)- silenced and normal cells with DLAT(PDH)-silenced using Metafectene® Pro (Biontex) for 24 h; the silencing efficiency of siRNAs was confirmed both by immmunoflorescence after 48 h in total.

Migration assay

For the interaction between the normal cell line MRC5 and the two lung cancer cell lines H1299 and A549 in migration ability, an IBIDI Single Culture-Insert (IBIDI GmbH) in a 35 mm μ-Dish was used. This migration monitoring system consists of two reservoirs separated by a 500 μm thick wall. Equal numbers of control, DLAT(PDH)-silenced, PDK-silenced and LDHA(V)-silenced $7x10^5$ cells/ml were added into the two reservoirs of the same insert and incubated at 37°C/5% CO_2.
After 24 hours, the insert was removed with caution creating a gap of 500 μm. The 35mm μ-Dish has been finally filled with complete DMEM growth medium and the migration was monitored by bright-field microscopy at specific time points. The images were analysed using TScratch software, developed by the Koumoutsakos group (CSE Lab), at ETH Zurich, the % of cells free area was calculated and plotted using the GraphPad Prism 5.01 statistical package (GraphPad Software Inc., USA). To abolish proliferation as a confusing factor in our migration experiments, cells were treated with Mitomycin C (KYOWA KIRIN) at 5μg/mL for 3h before the insert was removed.

Results

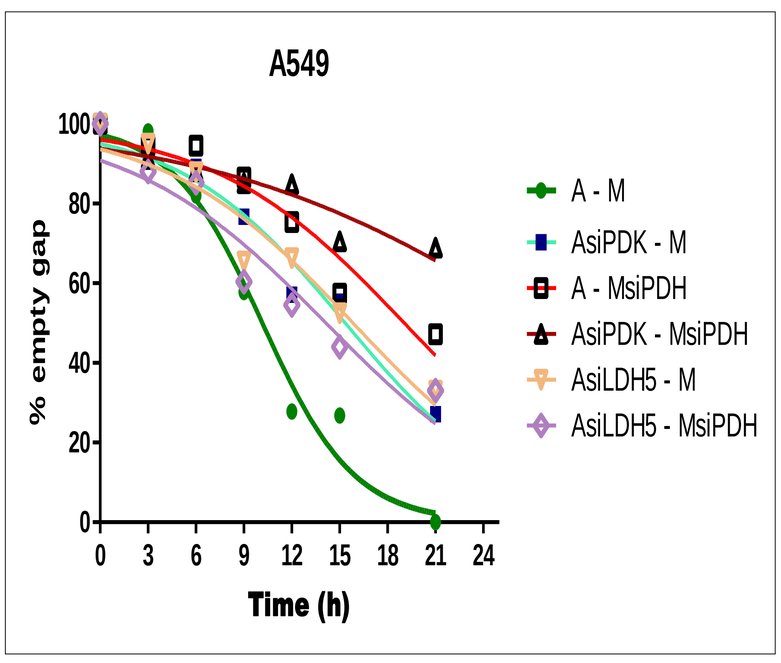

FIGURE 1. Inhibition of cell migration a) A(A549) and b) H (H1299) depending on the silencing of genes involved in anaerobic metabolism (LDH5, PKD1) or in aerobic metabolism of fibroblasts (PDH).

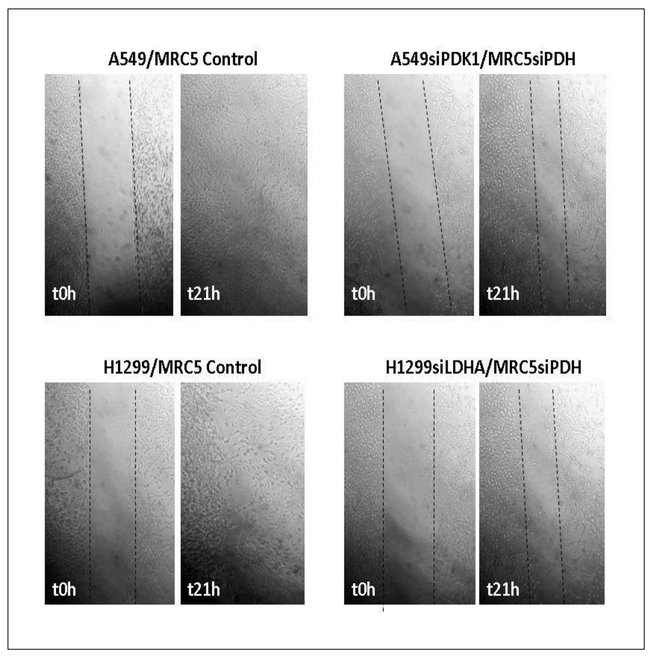

FIGURE 2 .Images of gap filling during cancer cells' migration to the fibroblasts' area.

Conclusions

The analysis of the filling gap speed between fibroblasts (MRC5) and cancer cells lines (A549 or H1299) showed that silencing of PDH gene in fibroblasts, or silencing of PDK and LDHA gene in tumor cells, or even simultaneous silencing of genes, leads to considerable delay of cancer cell migration. The A549 cancer cell migration inhibited at the maximum extent when in the cells was achieved simultaneous inhibition of the expression of PDH in fibroblasts and of PDK1 in tumor cells. In H1299 cell line maximal migration inhibition was achieved by the simultaneous inhibition of the expression of PDH in fibroblasts and LDH5 in tumor cells. The results supports the concept of a metabolic co-operation between lung cancer cells and fibroblasts that promotes cancer cell migration. This co-operation demands anaerobic metabolism of cancer cells, as shown by the critical role of the LDHA and PDK1 genes, and a functional Krebs cycle in fibroblasts, as interaction seems to demand intact PDH activity.

References

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