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14. ABSTRACT ERK signaling pathway plays a critical role in the survival and proliferation of invasive lung cancer cells. However, mechanism associated the regulation of high ERK activity in invasive lung cancer cells is not defined. This proposal is to test whether ERK activity is regulated by cellular cAMP concentration and particular cyclic nucleotide phosphodiesterase (PDE) isotype. In this study, we show that ERK activity is suppressed by raising cellular cAMP concentration in invasive lung cancers. qRT-PCR reveal that levels of PDE7B and PDE10A mRNA are elevated in invasive lung cancer cell lines in comparison with non-invasive lung cancer cell lines. However, only knockdown of PDE10A reduced ERK activity in invasive lung cancer cells. Moreover, we show that specific PDE10A inhibitor induced significant apoptosis in invasive lung cancer cells. Our study can thus provide a proof-of-concept of potentially using PDE inhibitor to treat metastatic lung cancers.					
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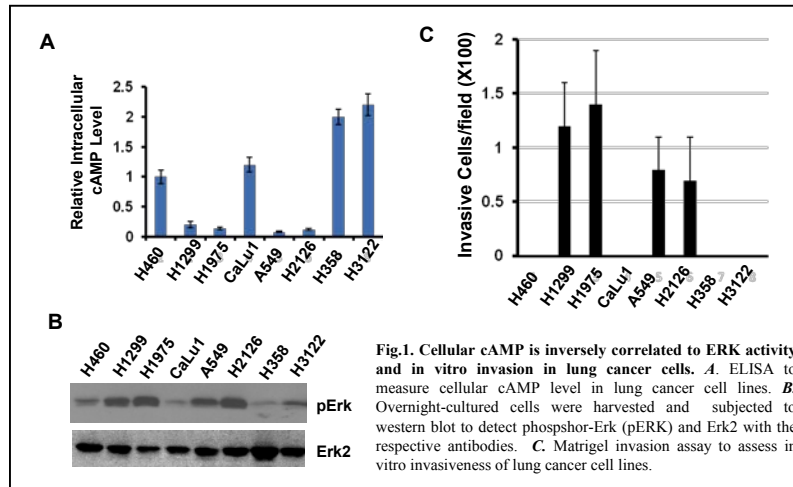
Introduction

Non-small cell lung cancer (NSCLC) kills approximately 140,000 people each year and diagnosed at advanced stages (IIIb and IV) in almost 50% of cases. Although chemotherapy prolongs the life of patients, severe side effects accompanying with chemotherapy often preclude advanced stage as well as senior patients receiving it. Recent advances in the understanding of the molecular mechanisms of lung cancers have brought the new hope on treatment of advanced NSCLCs. For example, ERGR and EML4-ALK inhibitors are effective with patients carrying specific mutation in their tumors. Unfortunately, only small percentage patients can benefit from these molecular therapies. Therefore, better understanding will undoubtedly help develop novel therapeutic approaches that can help those NSCLC patients not benefitting from the available agents.

ERK signaling pathway plays a critical role in the survival and proliferation of invasive lung cancer cells. It is thus expected that approaches interfering with ERK signaling pathway will lead to the suppression of lung cancer progression. Unfortunately, mechanism associated with the regulation of high ERK activity in invasive lung cancer cells is not understood. This proposal is to test our hypothesis that ERK activity is regulated by cellular cAMP concentration and particular cyclic nucleotide phosphodiesterase (PDE) isotype. In this study, we show that ERK activity is suppressed by raising cellular cAMP concentration in invasive lung cancers. Quantitative RT-PCR (qRT-PCR) further reveals that levels of PDE7B and PDE10A mRNA are elevated in invasive lung cancer cell lines in comparison with non-invasive lung cancer cell lines. However, only knockdown of PDE10A reduced ERK activity in invasive lung cancer cells. Moreover, we show that specific PDE10A inhibitor induced significant apoptosis in invasive lung cancer cells. We believe that this study has provided the basis of testing PDE10A inhibitor in lung cancer models.

Body

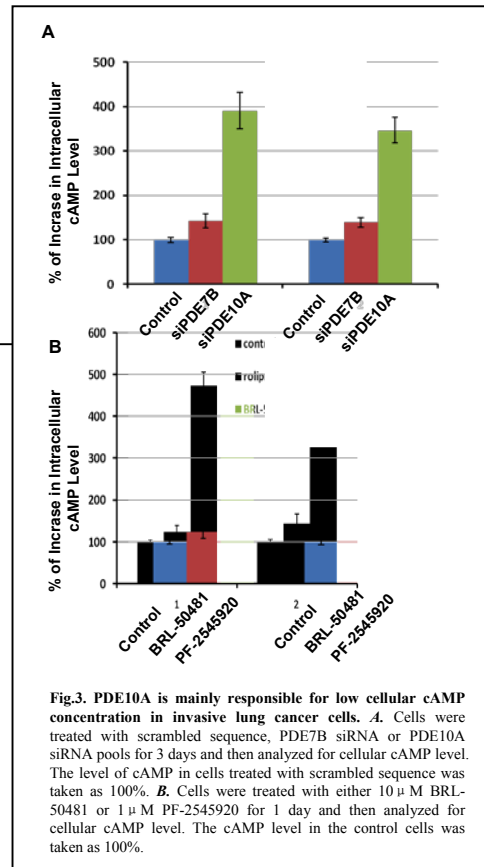
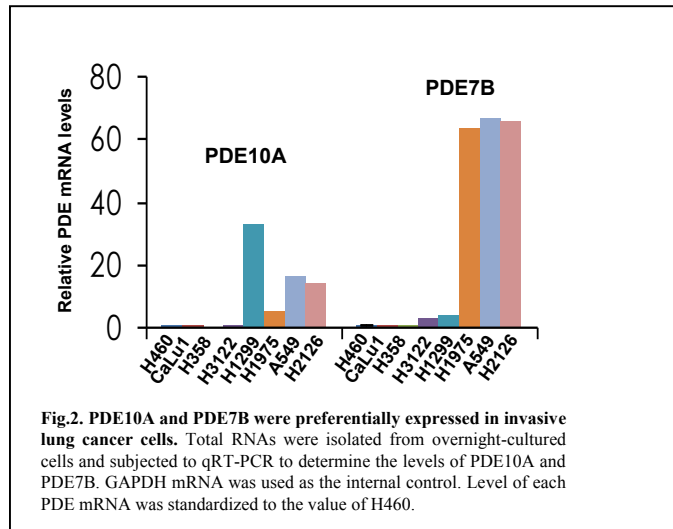
Tasks of this proposal are 1) Determine the relationship between Erk activity and intracellular cAMP concentration (months 1-4); 2) Characterize PDEs that can lower intracellular cAMP concentration in NSCLC cells (months 5-10); 3) Evaluate the effectiveness of specific PDE inhibitors to suppress growth/survival of NSCLC cells (months 11-12).



Task 1: We obtained 8 human NSCLC lines from ATCC and analyzed the activity of ERK (through the level of ERK phosphorylation by Western blot), cellular cAMP concentration (ELISA) and *in vitro* invasiveness (Matrigel invasion assay) (Fig.1). Clearly, lines displaying higher Erk activity have lower cellular cAMP

concentration and greater invasive capacity.

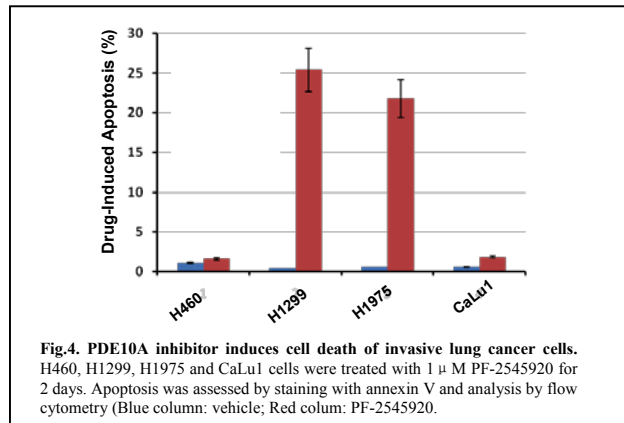
Task 2: Total RNA was isolated from these 8 cell lines and then subjected qRT-PCR with primer sets for each PDE isotypes (total 22 isotypes). Only PDE7B and PDE10A are consistently overexpressed in invasive lung cancer cell lines (A549, H1299, H1975 and H2126) (Fig.2). To determine whether reduced cellular cAMP



concentration and high Erk activity depends on

PDE7B or PDE10A, we delivered PDE7B or PDE10A siRNAs into H1299 and H1975 cells. Knockdown of PDE10A, but not PDE7B, led to increased cellular cAMP concentration and decreased ERK phosphorylation (Fig.3A). Treatment of PDE10 inhibitor also elevated cellular cAMP concentration and inhibited ERK activity in these two cell lines (Fig.3B).

Task 3: To determine the effect of blocking PDE10 activity on invasive NSCLC growth,



we treated invasive H1299, H1975 cells and non-invasive H460, CaLu1 with PDE10A inhibitor PF-2545920. MTT assay showed that PF-2545920 dose-dependently inhibited cell growth in invasive lines but not non-invasive lines (data not shown). In subsequent experiments, we performed Br-dU assay to assess cell proliferation and Annexin V staining to analyze apoptosis. While PF-2545920 did not significantly alter cell proliferation in all four lines (data not shown), it

induced apoptosis in invasive cell lines (Fig.4).

Key Research Accomplishment

We found that 1) cellular cAMP concentration is in an inverse correlation with ERK activity and *in vitro* invasion; 2) Suppressing PDE10A expression or activity leads to growth inhibition of NSCLC cells.

Reportable Outcomes

Our study suggests the possibility of specifically killing lung cancer cells by targeting PDE10A.

Conclusions

Cellular cAMP level is inversely correlated with ERK activity and *in vitro* invasion. Targeting PDE10A can reduce cellular cAMP concentration and induce cell death. Since PDE10A inhibitors are already in clinical trial for treating Huntington's Disease, our study has provided the "proof-of-concept" for further testing the usefulness of PDE10A inhibitor in lung cancer therapy.

References

None

Appendices

N/A