Label-free quantitative proteomic studies of A549 cells after prolonged nicotine exposure

Sasikarn Komkleow[1], Churat Weeraphan[2], Daranee Chokchaichamnankit[2], Papada Chaisuriya[2], Chris Verathamjamras[2], Theetat Ruangjaroon[2], Jisnuson Svasti[2,3], Polkit Sangvanich[4], and Chantragan Srisomsap[2,*]

[1]Program in Biotechnology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, [2]Laboratory of Biochemistry, Chulabhorn Research Institute, Bangkok, Thailand

[3]Applied Biological Sciences Program, Chulabhorn Royal Academy, Bangkok, Thailand, [4]Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

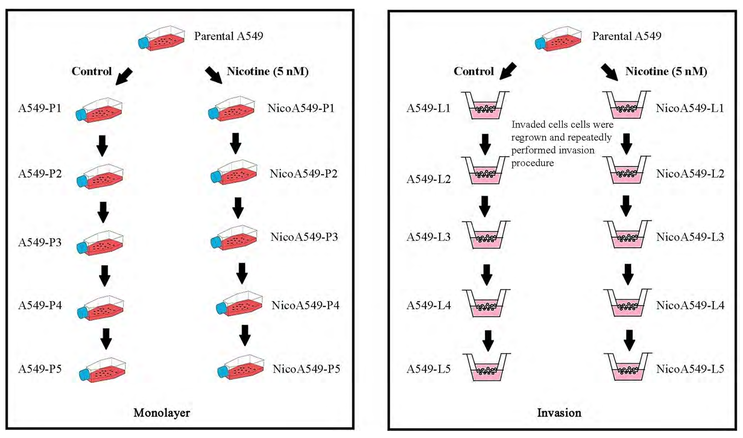
Introduction

Lung cancer is the most common cause of cancer-related death worldwide [1,2]. In Thailand, lung cancer accounts for 14.1% of new cancer cases in 2018 which is the highest frequency among other cancer types [1]. Although the diagnostic and treatment methods have been improved, the overall survival rate of lung cancer patient remains very poor with 5-year survival rate < 15% [3]. Approximately 80% of lung cancer deaths are caused by smoking. Lung cancer risk is therefore many times higher in smokers as compared to those in non-smokers [4].

Nicotine, an addictive component in cigarettes, is generally considered as non-carcinogenic. However, growing evidence indicates that prolonged nicotine exposure is a potential factor associated with tumorigenesis. Therefore, there is a need to gain insight into the molecular events during prolonged exposure to nicotine. Here, the effect of prolonged nicotine exposure on A549 lung adenocarcinoma cells was investigated, using label-free quantitative proteomic analysis. Selection of invasive subpopulation from A549 cell line was performed to reveal the differential expression and functional annotation of proteins in relation to prolonged nicotine exposure, using Boyden chamber assays in combination with the proteomics approach.

Methodologies

01 Cell invasion assay
02 Prolonged nicotine exposure of A549 cells
03 Prolonged nicotine exposure on motile A549 cells
04 Label free LC-MS/MS analysis
05 Western blot analysis
06 Bioinformatics



Results

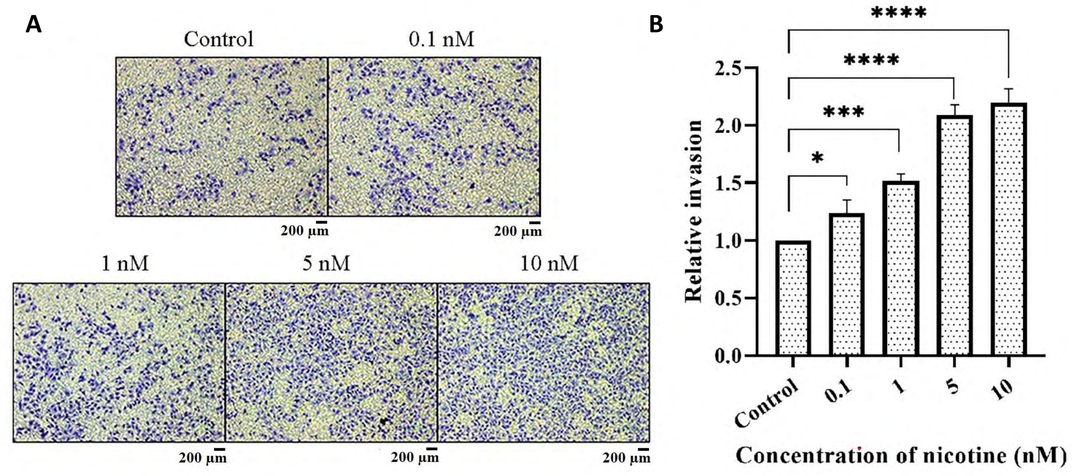


Figure 1. (A) Representative images of the invasion assay using A549 cells treated with 0.1, 1.0, 5, and 10 nM of nicotine for 24 h. Scale bar, 200 µm. (B) Bar graph represents the relative invasion of A549 cells after treatment with various concentrations of nicotine. Data points represent the mean±SD. (*p<0.05, ***p<0.001, ****p<0.0001).

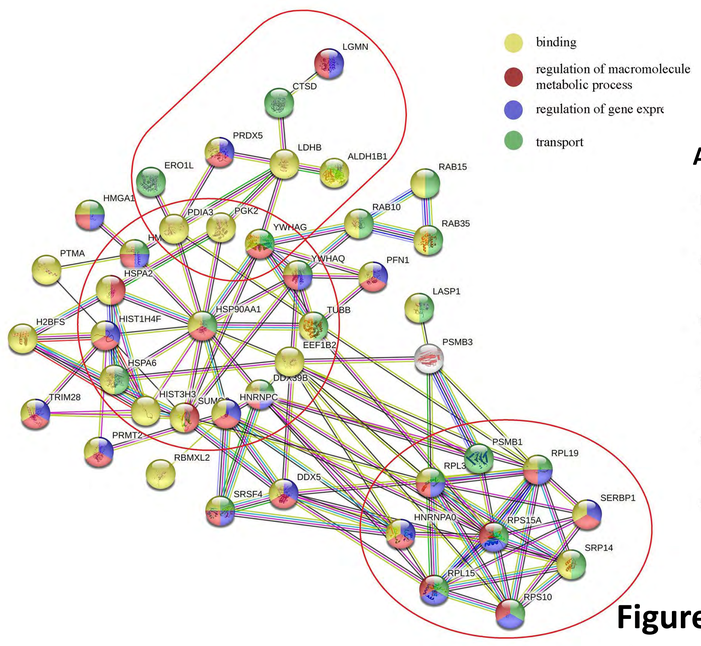


Figure 2. Nicotine induces differential protein expression in A549 cells growing as a monolayer culture (flask). The differentially expressed proteins were used to search the STRING database to predict their protein-protein interactions.

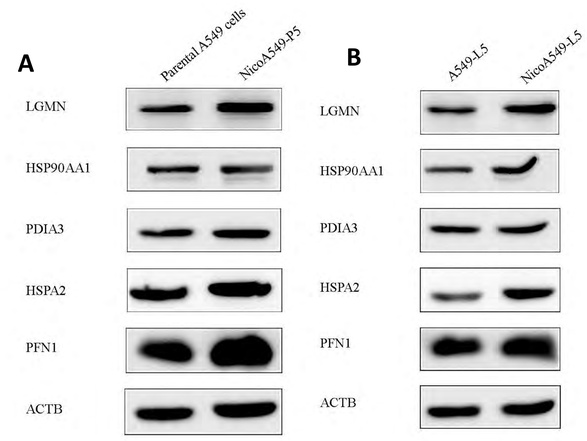


Figure 3. The expression levels of the dysregulated proteins in cells growing in monolayers and invasion system were verified by western blot analysis. (A) Western blot showing the expression of selected proteins in untreated and nicotine treated A549 cells from monolayer system. (B) Western blot showing the expression of selected proteins in untreated A549-L5 and nicotine treated A549-L5 cells from invasion system.

References

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Conclusions

- Nicotine induced A549 cell invasion in a dose-dependent manner
- Prolonged exposure of nicotine promoted invasion on A549 cells
- From LC-MS/MS, 55 proteins from monolayer system and 100 proteins from invasion system were identified with a change in expression level after prolonged nicotine treatment.
- The higher expression of legumain, heat shock protein HSP 90-alpha, heat shock-related 70 kDa protein 2, protein disulfide isomerase A3 and profilin-1 were observed in A549 cells after prolonged exposure to nicotine.
- These findings suggested that these proteins might serve as novel cancer biomarkers for cigarette smokers.

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