Calpeptin Induces Apoptosis in A549 Non-Small Cell Lung Cancer Cells

Chiharu Tabata (ctabata@huhs.ac.jp)
Hyogo University of Health Sciences
Rie Tabata
Saiseikai-Noe Hospital

Research Article

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Abstract

Lung cancer is a leading cause of cancer-related death worldwide, and most are non-small cell lung cancers (NSCLC). Since the overall survival remains very poor for NSCLC patients with advanced-stage disease, the development of novel treatments is needed. Previous studies reported a relationship between calpain and tumorigenesis. In this study, we examined the apoptotic effects of calpeptin (Cal), a calpain inhibitor, on A549 NSCLC cells. We assessed whether Cal induced apoptosis in A549 cells. Cal induced apoptosis in A549 cells and also activated p38MAPK. These results suggest a possible clinical use of Cal for the treatment of NSCLC.

Introduction

Lung cancer is a leading cause of cancer-related death worldwide and is categorized into two histological subtypes; small cell lung cancer and non-small cell lung cancer (NSCLC). Furthermore, NSCLC are mainly adenocarcinomas. Although several treatments, such as platinum doublet chemotherapy and molecular target therapy, are clinically used for the treatment of NSCLC, the overall survival of NSCLC patients with advanced-stage disease or metastatic lesions remains very poor [1, 2]. Therefore, the development of novel treatments for NCSLC is needed.

Calpain is a calcium-dependent intracellular cysteine protease, and the mammalian calpain family comprises of fifteen gene products. Classical calpains, such as calpain-1 and calpain-2, are ubiquitously expressed, whereas others are expressed in specific tissues such as the skeletal muscle and gastrointestinal tract. Calpain-1 and calpain-2 are also referred to as μ-calpain and m-calpain, respectively, because they require specific calcium concentrations (in μM and mM amounts, respectively) for their activation. Calpain plays important roles in various cellular processes that include cell growth and cellular signaling [3]. Previous studies reported a relationship between calpain and tumorigenesis [4] in cancers such as schwannomas [5], colon cancer [6] and renal cell carcinoma [7].

We previously reported that calpeptin (Cal), an inhibitor of calpain-1 and calpain-2, prevents A549 NSCLC cell proliferation [8]. However, the relationship between calpain and the apoptosis of lung cancer cells have not yet been studied in detail. Therefore, we assessed whether calpeptin (Cal), an inhibitor of calpain-1 and calpain-2, exert apoptosis-inducing effects in A549 NSCLC cells.

Materials And Methods

Cell culture

A549 cells, a human NSCLC cell line, were cultured in Dulbecco's modified Eagle's medium (DMEM) (Sigma Chemical Co., St Louis, MO) supplemented with 10% heat-inactivated fetal calf serum in a humidified incubator with 5% CO_2 at 37^{o}C. Cal (Calbiochem, San Diego, CA) was diluted in DMSO and added to the growth medium to yield a final DMSO concentration of < 0.01% (v/v). For the control, the cells without Cal were treated with the same concentration of DMSO. All cultures in this study contained

the same final concentration of DMSO. In preliminary experiments, the final concentration of DMSO had no marked effects on A549 cells.

Cell apoptosis assay

A549 cells were cultured with or without 100 nM Cal for 72 hours. After treatment, cytoplasmic extracts were prepared using the Nuclear Extract Kit (Active Motif, Carlsbad, CA), and protein concentrations in the cytoplasmic extracts were measured as previously described [9, 10]. Cell apoptosis was evaluated using Cell Death Detection ELISA^{PLUS} (Roche, Mannheim, Germany) according to the manufacturer's instructions.

Measurement of p38MAPK

A549 cells were cultured with or without 100 nM Cal for 1 hour. After treatment, cytoplasmic extracts were prepared and protein concentrations in the extracts were measured as previously described [9, 10]. Cytoplasmic phospho-p38MAPK (pThr^{180}/pThr^{182}) and ERK1/2 were detected using an ELISA Kit (Sigma) as previously described [10].

Statistical analysis

The results are shown as the mean ± SD. Statistical analyses were performed using the Bonferroni-Dunn multiple comparisons test.

Results

Cal-induced apoptosis effects in A549 cells

We examined the effects of Cal on apoptosis in A549 cells. Apoptosis in A549 cells was induced by treatment of Cal after 72 h of culture ($p<0.01$) (Fig. 1A).

P38mapk Is Involved In Cal-induced Apoptosis In A549 Cells

In order to understand the mechanism underlying the induced effect of Cal on apoptosis in A549 cells, we examined whether any kinases are required for this process. We showed that the levels of cytoplasmic p38MAPK in cells were activated in the presence of Cal after 1 h of cultivation ($p< 0.01$, Fig. 1B). Cellular levels of cytoplasmic ERK1/2 were not affected by Cal (data not shown).

Discussion

In this study, we investigated the relationship between Cal and apoptosis in A549 cells and found that Cal induced apoptosis in these cells. We then attempted to elucidate the mechanism underlying the induction

of A549 cell apoptosis by Cal.

The MAPK signaling pathway plays an important role in major cellular processes including proliferation and apoptosis. MAPKs consist of kinases such as p38MAPK and ERK1/2 [11, 12], and the former is involved in inducing apoptosis [13]. Kong et al. reported that the activation of p38MAPK and suppression of ERK1/2 plays a central role in the apoptosis of diffuse large B cell lymphoma cells [14]. Furthermore, Ye et al. showed the induction of apoptosis by p38MAPK activation in A549 NSCLC cells [15]. In this study, we examined the relationship between Cal and MAPKs and showed that the levels of cytoplasmic p38MAPK, but not ERK1/2, in A549 cells were activated in the presence of Cal.

Idiopathic pulmonary fibrosis (IPF) occasionally occurs in patients with lung cancer (largely NSCLC) because of smoking history or complication such as emphysema [16–18]; however, the mechanism by which it occurs remains mostly unknown. Chemotherapy or radiation therapy are effective for NSCLC, however these therapies occasionally induce pulmonary fibrosis and so these are not selectable for NSCLC complicated with pulmonary fibrosis.

We previously reported that Cal prevented bleomycin-induced pulmonary fibrosis in mice [19]. Cal also decreases the expression of IL-6, TGF-β_1, angiopoietin-1, and collagen type Iα1 mRNA in mouse lung tissues. *In vitro* studies showed that Cal decreased the production of IL-6, TGF-β_1, and angiopoietin-1 as well as the synthesis of collagen by lung fibroblasts. Furthermore, Cal decreased IL-6-dependent proliferation and angiopoietin-1-dependent migration of lung fibroblasts. These may be possible mechanisms by which Cal suppresses pulmonary fibrosis.

We previously reported that Cal prevents A549 NSCLC cell proliferation [8]. Moreover, we showed that Cal induced apoptosis in A549 cells in this study, suggesting its clinical application in patients with NSCLC complicated with IPF.

In summary, we demonstrated the apoptosis-inducing effects of Cal in A549 cells which may have been due to the activation of p38MAPK. Although the precise cellular mechanism underlying the Cal-induced apoptosis of A549 cell is not fully understood, our results may lead to the development of novel strategies for the treatment of NSCLC.

Abbreviations

Cal
Calpeptin
NSCLC
non-small cell lung cancer
IPF
idiopathic pulmonary fibrosis

Declarations

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Contributions: Tabata C and Tabata R designed the study. Tabata C performed the research. Tabata C collected the data. Tabata C and Tabata R analyzed and interpreted the data. Tabata C performed the statistical analysis. Tabata C, Tabata R wrote the manuscript.

References

1. Goldstraw, P. *et al.* Non-small-cell lung cancer., **378** (9804), 1727–1740 (2011).
2. Reck, M., Heigener, D. F., Mok, T., Soria, J. C. & Rabe, K. F. Management of non-small-cell lung cancer: recent developments., **382** (9893), 709–719 (2013).
3. Goll, D. E., Thompson, V. F., Li, H., Wei, W. & Cong, J. The calpain system. *Physiol Rev*, **83**, 731–801 (2003).
4. Storr, S. J., Carragher, N. O., Frame, M. C., Parr, T. & Martin, S. G. The calpain system and cancer. *Nat Rev Cancer*, **11** (5), 364–374 (2011).
5. Kimura, Y. *et al.* The involvement of calpain-dependent proteolysis of the tumor suppressor NF2 (merlin) in schwannomas and meningiomas. *Nat Med*, **4** (8), 915–922 (1998).
6. Lakshmikuttyamma, A., Selvakumar, P., Kanthan, R. & Kanthan, S. C. Sharma RK Overexpression of m-calpain in human colorectal adenocarcinomas. *Cancer Epidemiol Biomarkers Prev***13**(10),1604–9, 2004.
7. Braun, C. *et al.* Expression of calpain I messenger RNA in human renal cell carcinoma: correlation with lymph node metastasis and histological type. *Int J Cancer*, **84** (1), 6–9 (1999).
8. Tabata, C., Tabata, R. & Nakano, T. Calpeptin Prevents angiopoietin-1-induced proliferation of non-small cell lung cancer A549 cells. *Int J Clin Exp Pathol*, **9**, 3672–3677 (2016).
9. Tabata, C. *et al.* All-trans retinoic acid modulates radiation-induced proliferation of lung fibroblasts via IL-6/IL-6R system. *Am J Physiol Lung Cell Mol Physiol*, **290**, 597–606 (2006).
10. Tabata, C. *et al.* All-trans-Retinoic Acid Prevents Radiation- or Bleomycin-induced Pulmonary Fibrosis. *Am J Respir Crit Care Med*, **174**, 1352–1360 (2006).
11. Cobb, M. H. MAP kinase pathways. *Prog Biophys Mol Biol*, **71** (3-4), 479–500 (1999).
12. Pearson, G. *et al.* Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev*, **22** (2), 153–183 (2001).
13. Xia, Z., Dickens, M., Raingeaud, J., Davis, R. J. & Greenberg, M. E. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis., **270** (5240), 1326–1331 (1995).

14. Kong, Y. *et al.* Pterostilbene induces apoptosis and cell cycle arrest in diffuse large B-cell lymphoma cells. *Sci Rep*, **6**, 37417 (2016).
15. Ye, B. *et al.* Anti-tumor activity of CrTX in human lung adenocarcinoma cell line A549. *Acta Pharmacol Sin*, **32** (11), 1397–1401 (2011).
16. Bouros, D., Hatzakis, K., Labrakis, H. & Zeibecoglou, K. Association of malignancy with diseases causing interstitial pulmonary changes., **121** (4), 1278–1289 (2002).
17. King, T. E. Jr, Pardo, A. & Selman, M. Idiopathic pulmonary fibrosis., **378** (9807), 1949–1961 (2011).
18. Karampitsakos, T. *et al.* Lung cancer in patients with idiopathic pulmonary fibrosis. *Pulm Pharmacol Ther*, **45**, 1–10 (2017).
19. Tabata, C., Tabata, R. & Nakano, T. The calpain inhibitor calpeptin prevents bleomycin-induced pulmonary fibrosis in mice. *Clin Exp Immunol*, **162** (3), 560–567 (2010).

Figures

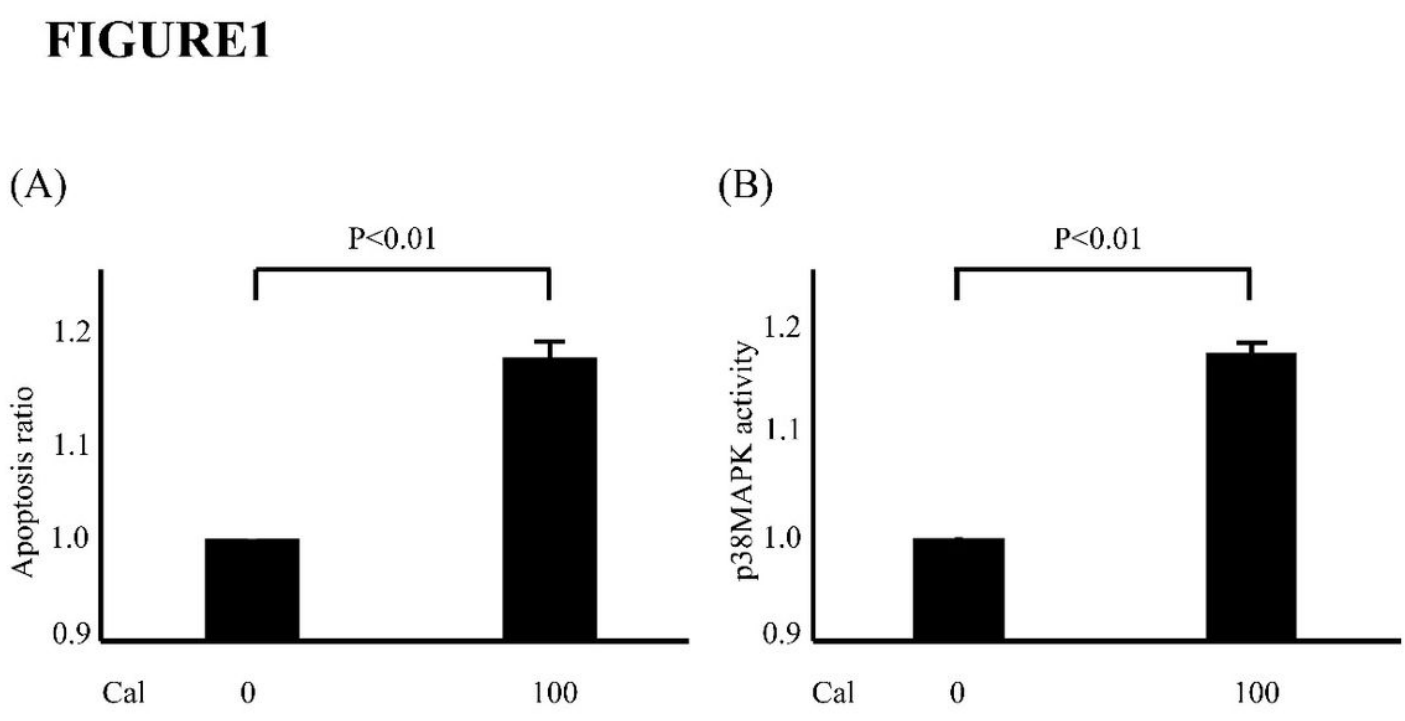

Figure 1

Effects of Cal on apoptosis and p38MAPK activity in A549 cells. A549 cells were cultured with or without Cal (100 nM) for 72 hours (A) and 1 hour (B). A cell apoptosis assay was performed (A). The p38MAPK

activity in cytoplasmic extracts was analyzed (B). The levels were corrected based on the protein concentration of each sample. The values were normalized to the control (without calpeptin) and are presented as a ratio. All results are shown as the mean ± SD.