Polypeptide Fibrillar Assemblies Exhibit Membranolytic Effects and Anti-Metastatic Activity on Lung Cancer Cells

Yu-Fon Chen, Chien-Hsiang Chang, Ming-Wei Hsu, Ho-Min Chang, Yi-Cheng Chen, Yi-Sheng Jiang and Jeng-Shiung Jan*

Corresponding Author

Jeng-Shiung Jan-Department of Chemical Engineering, National Cheng Kung University, No. 1 University Rd., Tainan, 70101 Taiwan. Email: jsjan@mail.ncku.edu.tw, Phone: +886-6-2757575 ext. 62660, Fax: +886-6-2344496

Authors

Yu-Fon Chen-Department of Chemical Engineering, National Cheng Kung University, No. 1 University Rd., Tainan, 70101 Taiwan
Chien-Hsiang Chang-Department of Chemical Engineering, National Cheng Kung University, No. 1 University Rd., Tainan, 70101 Taiwan
Ming-Wei Hsu-Department of Medicine, College of Medicine, National Cheng Kung University, No. 1 University Rd., Tainan, 70101 Taiwan
Ho-Min Chang-Department of Chemical Engineering, National Cheng Kung University, No. 1 University Rd., Tainan, 70101 Taiwan
Yi-Cheng Chen-Department of Biochemistry and Molecular Biology, College of Medicine, National Cheng Kung University, No. 1 University Rd., Tainan, 70101 Taiwan
Yi-Sheng Jiang-Department of Chemical Engineering, National Cheng Kung University, No. 1 University Rd., Tainan, 70101 Taiwan
Materials

Hydrogen bromide, iodotrimethylsilane, trifluoroacetic acid-$d_1$ (99.5%), D$_2$O (99.9%), β-actin antibodies, DAPI, and Rhodamine 123 (Sigma-Aldrich); FBS and DMEM (Invitrogen); A549, H1299, NIH3T3, and LL2 cells (Bioresource Collection and Research Center, Taiwan); NOD-SCID mice (National Laboratory Animal Center, Taiwan); Cell Counting Kit 8/CCK-8 reagent (Dojindo); D-luciferin potassium salt (Promega); Human cyt c ELISA Kit (BioVision); JC1-Mitochondrial membrane potential assay kit (Abcam); Mitochondria/cytosol fractionation Kit (Abcam); Apoptosis-inducing factor and endonuclease G antibodies (Santa Cruz); Caspase-3/8/9 antibodies (Cell Signaling Technology); Goat anti-mouse/goat anti-rabbit HRP-linked secondary antibodies (Jackson ImmunoResearch); PVDF membranes and ECL kit (Pierce Biotechnology)
Table S1. Composition ratio, number average molecular weight ($M_n$), and molecular weight distribution ($M_w/M_n$) of PZLL-$b$-PBnT block copolypeptides.

| Polypeptide | Feed molar ratio $^{1}$H NMR GPC |
|-------------|-------------------|------------------|-------------------|
|              | Initiator:ZLL:BnT | Initiator:ZLL:BnT | $M_n$ | $M_w/M_n$ |
| PZLL$_{10}$-b-PBnT$_{20}$ | 1 : 10 : 20 | 1 : 11 : 19 | 6100 | 1.65 |
| PZLL$_{15}$-b-PBnT$_{15}$ | 1 : 15 : 15 | 1 : 17 : 15 | 6900 | 1.43 |
| PZLL$_{20}$-b-PBnT$_{10}$ | 1 : 20 : 10 | 1 : 20 : 10 | 6600 | 1.10 |
| PZLL$_{20}$-b-PBnT$_{20}$ | 1 : 20 : 20 | 1 : 20 : 18 | 8400 | 1.36 |
| PZLL$_{30}$-b-PBnT$_{15}$ | 1 : 30 : 15 | 1 : 30 : 14 | 10400 | 1.27 |
Figure S1. $^1$H NMR spectra of (a) PZLL$_{20}$ in TFA-$d_1$, (b) (Z-Lys)$_{20}$-$b$-(Bn-Thr)$_{10}$ in TFA-$d_1$, and (c) Lys$_{20}$-$b$-Thr$_{10}$ in D$_2$O.
Figure S2. $^1$H NMR spectra of (a) (Z-Lys)$_{10}$-b-(Bn-Thr)$_{20}$, (b) (Z-Lys)$_{15}$-b-(Bn-Thr)$_{15}$, (c) (Z-Lys)$_{20}$-b-(Bn-Thr)$_{10}$, (d) (Z-Lys)$_{20}$-b-(Bn-Thr)$_{20}$, and (e) (Z-Lys)$_{30}$-b-(Bn-Thr)$_{15}$ in TFA-$d_1$. 
Figure S3. CD spectrum of Lys_{20-20}b-Thr_{10} dissolved in PBS (0.125 mg/mL).
Figure S4. cryoEM image of Lys$_{20}$-b-Thr$_{10}$ PFA stained with uranyl acetate. As marked by the red circles, the worm-like structures can be seen in the image.
Figure S5. SAXS patterns of Lys\textsubscript{10}-b-Thr\textsubscript{20}, Lys\textsubscript{15}-b-Thr\textsubscript{15}, Lys\textsubscript{20}-b-Thr\textsubscript{10} and Lys\textsubscript{30}-b-Thr\textsubscript{15} PFAs in aqueous solutions (0.5 wt%).
Figure S6. Coil Lys_{20} and PFAs (Lys_{20}-b-Thr_{10}, Lys_{20}-b-Thr_{20} and Lys_{20}-b-Thr_{40}) induced membranolysis in A549 cells. (A-B) A549-luciferase cells were treated with or without polypeptides for 1 h. Luminescent images were taken a few minutes after treating with luciferin substrate and expressed as total photon flux. RLU was quantified using a luminescence image analyzer and normalized according to the saline (100 %) and 1% TritonX-100 (0 %) groups (n = 3). (B: two-way ANOVA)
Figure S7. Coil PLL (Lys20 and Lys60) and PFAs (Lys20-b-Thr10, Lys20-b-Thr20, Lys20-b-Thr40 and Lys40-b-Thr20) and coil induced caspase-dependent apoptosis in H1299 cells. Cells were treated with or without various concentrations of polypeptides for 1 h, and total cell lysates were subjected to immunoblotting for detecting caspase/cleaved caspase-3, 8 and 9. Histograms represent the relative expression levels quantified using densitometric analysis with ImageJ software and normalized according to the β-actin reference bands. The * and # symbols represent statistical significances of procaspase-8/procaspase-3- vs. procaspase-9/caspase-3-treated groups. Similar results were obtained in three independent experiments. The statistical analysis (Unpaired Student’s t test) compares treatment groups with control groups.
Coil Lys<sub>20</sub>, Thr<sub>10</sub> and PFAs (Lys<sub>10</sub>-b-Thr<sub>20</sub>, Lys<sub>15</sub>-b-Thr<sub>15</sub> and Lys<sub>20</sub>-b-Thr<sub>10</sub>) induced mitochondrial membrane depolarization. H1299 cells (1×10<sup>4</sup> cells/well) were cultured on 96-well plates and stained with 20 μM of JC-1 dye-loading solution followed by incubation for 10 min at 37°C. After washing twice, cells were treated with the polypeptides above (5 μM) for 30 min. The change of mitochondrial membrane potential was measured as the ratio between aggregate (Em=590 nm) and monomeric forms (Em=530 nm) of JC-1. Decreasing ratios indicate mitochondrial membrane depolarization. FCCP (100 μM) was used as the depolarization control. The * and # symbols represent the statistical significances of the polypeptide/FCCP- vs. vehicle-treated groups and the polypeptide/FCCP- vs. Lys<sub>20</sub>-treated groups, respectively. All data were statistically analyzed using an Unpaired Student’s t test (n = 3; *p < 0.05, **p < 0.01, and ***p < 0.001).