Identification of actin as a direct proteomic target of berberine using an affinity-based chemical probe and elucidation of its modulatory role in actin assembly†

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Despite the diverse pharmacological activities of berberine, including anti-cancer and anti-inflammatory effects, the direct proteomic targets of berberine have remained largely unknown. Here, we have identified actin as a direct proteomic target of berberine using an affinity-based chemical probe. In addition, we found that actin assembly was significantly modulated by berberine in vitro at the biochemical level and cellular level.

Berberine, one of the active ingredients found in many medicinal plants including Berberis vulgaris, is a natural benzylisoquinoline alkaloid.1–3 It shows various biological activities, especially anti-cancer effects.4–17 Driven by various reports about the pharmacological activities of berberine, tremendous efforts have been made toward its clinical use, but further development into novel drug candidates has been relatively limited, partly due to insufficient information about berberine's direct targets and mode of action. Recently, several studies have reported that berberine could bind and stabilize human telomeric G–quadruplex structures, formed at the single-stranded overhang of telomeric DNA, leading to potent anticancer activity in cancerous cells.18–20 Given that berberine has various biological activities and its complex formation with telomeric G–quadruplex DNA can explain only a small amount of its anti-cancer activity, it is assumed that another target might be associated with its various pharmacological activities including anti-cancer effects and further efforts to systematically investigate the cellular targets of berberine are significantly needed. To date, a direct proteomic target of berberine has scarcely been explored, although its complexation with DNA has been studied as a mode of its action.21 In recent decades, affinity-based chemical probes have been widely used and considered as powerful tools for identifying proteomic targets of biologically active small molecules.22–25 In an effort to find the proteomic target of berberine, we considered it worthwhile to synthesize and use an affinity-based chemical probe. In this study, we synthesized an affinity-based chemical probe of berberine and thus directly pulled down the cellular proteins, leading to identification of the molecular target by mass analysis. We also showed that the identified target can be modulated by berberine in vitro at the biochemical level and cellular level.

Based on a previous study on its structure–activity relationship,26 focusing on the anti-cancer activity of berberine, we designed and synthesized the chemical probe berberine biotinylated probe (BBP, 4), composed of berberine, the hydrophilic linker from the C-9 position of berberine, and biotin (Scheme 1). BBP (4) was concisely and efficiently synthesized via 2 step sequences from known compound 1,26 which was easily converted into compound 2 by simple propargylation of the phenol moiety without a base. BBP (4) was finally obtained through

Scheme 1 Synthesis of probe 4: (a) propargyl bromide, DMF, 80 °C, 100%; and (b) 3, CuSO4, Na ascorbate, t-BuOH, H2O, rt, 28%.
click chemistry of compound 2 with commercially available azido biotin (3) in the presence of CuSO₄ and sodium ascorbate.

Using synthesized BBP (4), we performed the target identification process on berberine. The overall process of target identification is briefly described in Fig. 1A. The proteome in a whole cell lysate of Jurkat T-cells was treated with BBP (20 μM) and incubated for 2 hours with or without free berberine (40 μM). Via enrichment with streptavidin-resin and a sequential wash-out process, the proteome labeled with BBP (4) was separated out by an insoluble solid support and isolated from the obtained proteome mixture. Then, proteome labeled with BBP or biotin-azide was released from the streptavidin bead and run on an SDS-PAGE gel, followed by silver staining. As shown in Fig. 1B, a protein band around 40 kDa in size was detected from the sample co-precipitated with BBP, suggesting its specific interaction with BBP. Moreover, the band intensity was drastically decreased in the presence of free berberine, indicating a specific interaction between the corresponding protein and berberine. Mass-spectrometry (MS/MS) analysis of the excised band identified the peptides covering 25.07% of protein and berberine. Mass-spectrometry (MS/MS) analysis of the excised band identified the peptides covering 25.07% of protein and berberine. Mass-spectrometry (MS/MS) analysis of the excised band identified the peptides covering 25.07% of protein and berberine. Mass-spectrometry (MS/MS) analysis of the excised band identified the peptides covering 25.07% of protein and berberine. Mass-spectrometry (MS/MS) analysis of the excised band identified the peptides covering 25.07% of protein and berberine. Mass-spectrometry (MS/MS) analysis of the excised band identified the peptides covering 25.07% of protein and berberine. Mass-spectrometry (MS/MS) analysis of the excised band identified the peptides covering 25.07% of protein and berberine.

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Considering our results and previous reports showing that berberine inhibits cancer cell migration and metastasis, we assumed that these anti-cancer properties might be associated with an inhibitory role of berberine in actin assembly.27 Therefore, we examined whether berberine could affect the in vitro actin polymerization process. Assembly of monomeric pyrene G-actin into filamentous F-actin was monitored by measuring pyrene fluorescence.28 As depicted in Fig. 2A, the addition of berberine resulted in dose-dependent inhibition of actin polymerization, suggesting that berberine is capable of directly suppressing the actin assembly process through direct interaction. Furthermore, disassembly of pre-assembled filamentous actin was also dramatically facilitated by the addition of berberine in a dose-dependent manner (Fig. 2B). This might be due to a shift from the dynamic equilibrium state to the disassembly process by suppression of actin polymerization by berberine.

Next, we used a macrophage podosome reformation assay to determine whether berberine can inhibit actin polymerization at the cellular level. Starved RAW macrophage cells were incubated with or without berberine for 3 hours and treated with PP2 (CAS 172889-27-9) to disrupt existing podosomes, followed by washing and incubation in serum-containing media for 30 minutes to allow podosome reformation. As shown in Fig. 3A, berberine treatment resulted in a significant decrease in podosome formation, further confirming the inhibitory role of berberine in actin assembly.27 To examine the effect of berberine on actin architecture in cells, NIH3T3 cells were briefly treated with a low concentration of Triton X-100 (0.0025%, 10 min, 4 °C) to increase the cell permeability and then treated with a vehicle or berberine. Three hours after treatment, fragmented actin filaments were

![Fig. 1](image1.png) Identification of actin as a direct interacting protein of berberine (BBR). Panel A shows the scheme of affinity purification of berberine interacting proteins and the proteomic identification process by LC-MS/MS. Panel B shows silver stained proteins co-precipitated with BBP. Panel C shows the peptides of actin (red) detected by LC-MS/MS analysis. Panel D shows the result of the direct pull-down assay using purified actin and BBP. Panel E shows SPR sensograms obtained on an actin coated chip at different concentrations of BBR.

![Fig. 2](image2.png) Modulation of in vitro actin assembly by berberine. Panel A shows the inhibition of pyrene-actin polymerization by berberine (BBR). Panel B shows the induction of pyrene-actin depolymerization by berberine (BBR). Polymerization and depolymerization of pyrene actin were analyzed by monitoring fluorescence signals.
increased in berberine (100 μM) treated cells as analyzed by Alexa Fluor 594 phalloidin staining. The cells treated with berberine for 48 hours showed significantly disrupted actin filament networks compared to vehicle treated cells (Fig. 3B).

In summary, we identified actin as a potential direct target of berberine using an affinity-based probe and elucidated that berberine is capable of modulating actin polymerization by berberine may provide a reasonable explanation for its biological activities such as inhibition of cell migration and cancer cell invasion. This result provided that berberine could directly bind to actin, which has been a crucial target for metastatic cancers, implying that berberine would serve as a useful tool for biological sciences related in its pharmacology and novel scaffolds for development of anti-cancer drugs.

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Notes and references