Biochemical and Biophysical Research Communications xxx (xxxx) xxx

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

A novel celecoxib analog UTX-121 inhibits HT1080 cell invasion by modulating membrane-type 1 matrix metalloproteinase

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ARTICLE INFO

Article history:
Received 25 September 2019
Accepted 10 October 2019
Available online xxx

Keywords:
COX
MMP
MT1-MMP
Invasion
Cell adhesion

ABSTRACT

We designed and synthesized a celecoxib derivative UTX-121 to enhance its anti-tumor activity. Similar to celecoxib, this compound could also inhibit matrix metalloproteinase (MMP)-9 activity. In addition, UTX-121 suppressed membrane-type 1 MMP (MT1-MMP)-mediated pro-MMP-2 activation by disturbing the cell surface expression of MT1-MMP. UTX-121 also impeded the glycosylation of cell surface proteins, resulting in the suppression of cell attachment to fibronectin. This inhibition by UTX-121 caused the reduction of fibronectin-stimulated focal adhesion kinase activation, Akt activation, and cell migration. Consequently, UTX-121 treatment significantly inhibited fibronectin-induced HT1080 cell invasion into the Matrigel. UTX-121 may be a potent lead compound that can be used to develop a novel anti-tumor drug.

1. Introduction

Celecoxib, a nonsteroidal anti-inflammatory drug, is a selective cyclooxygenase (COX)-2 inhibitor. COX enzymes, COX-1 and COX-2, are involved in the synthesis of prostaglandins (PG), which are autocrine mediators of multiple cellular processes. COX-1 is constitutively and ubiquitously expressed under basal conditions; however, COX-2 is only rapidly and transiently expressed in response to extracellular or intracellular stimuli, such as mitogens, growth factors, cytokines, and infectious agents. Therefore, COX-2 has been implicated in various pathologies, such as inflammation and carcinogenesis, by activating downstream PGE_2 signaling [1]. Aberrant COX-2 expression has been observed in many cancers and is associated with poor prognosis, particularly in colon and breast cancer [1–3]. Deregulation of COX-2/PGE_2 signaling activates mitogen-activated protein kinase (MAPK) and phosphatidylinositide 3-kinase (PI3K)/Akt pathways, which can induce COX-2 expression as a positive-feedback loop [1,4,5].

Many studies have reported on the anti-tumor activities of celecoxib, including anti-angiogenesis activity, apoptosis-induction activity, and anti-epithelial–mesenchymal transition properties [5]. Celecoxib treatment significantly reduces the number of colorectal polyps in patients with familial adenomatous polyposis [6]. However, these anti-tumor activities of celecoxib are only observed at higher concentrations than those needed to inhibit PG synthesis. Moreover, celecoxib suppresses tumor growth in the absence of any apparent COX-2 involvement [7]. These findings indicate that celecoxib exerts its anti-tumor activity through both COX-2-dependent and COX-2-independent mechanisms [8]. However, the COX-2-independent pharmacologic activities and mechanisms of celecoxib are unclear thus far.

Matrix metalloproteinases (MMPs), a family of Zn^{2+}-dependent enzymes that are essential for extracellular matrix (ECM) remodeling, are involved in many biological processes such as angiogenesis, inflammation, and cancer [9]. Membrane-type 1 MMP (MT1-MMP) is considered to play a crucial role in tumor progression as its expression most closely correlates with the invasive

Abbreviations: COX, cyclooxygenase; DMEM, Dulbecco's modified Eagle's medium; ECM, extracellular matrix; FAK, focal adhesion kinase; FBS, fetal bovine serum; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; MT1-MMP, membrane-type 1 MMP; PBS, phosphate-buffered saline; PG, prostaglandin; PI3K, phosphatidylinositide 3- kinase.

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https://doi.org/10.1016/j.bbrc.2019.10.092
0006-291X/

phenotype of malignant tumors among MMPs. Furthermore, MT1-MMP inhibition suppresses tumor cell invasion both *in vitro* and *in vivo*. Originally, MT1-MMP was identified as a tumor-specific latent MMP-2 (pro-MMP-2) activator [10]. It activates pro-MMP-9 and pro-MMP-13 and degrade a variety of ECM components, including type I, II, and III collagen and fibronectin. Besides ECM degrading activity, this enzyme cleavages cell adhesion molecules, cytokines, and others [11].

Celecoxib reduces PGE_2 and MMP expression in many types of cells, including macrophages, fibroblasts, and tumor cells [12,13]. This study aimed to evaluate whether the anti-metastatic activity of the newly designed and synthesized celecoxib analog UTX-121 can enhance COX-2-independent anti-tumor activity.

2. Materials and methods

2.1. Synthesis of UTX-121

(Supplementary methods).

2.2. COX-2 inhibition assay

(Supplementary methods).

2.3. Cell culture and materials

HT1080 human fibrosarcoma cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and were maintained in Dulbecco's modified Eagle medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS). Type I collagen was purchased from Nitta Gelatin (Osaka, Japan). Fibronectin (FN) was obtained from Asahi Techno Glass (Tokyo, Japan). The synthetic MMP inhibitor BB94 was gifted by Kotobuki Pharmaceutical Co. (Nagano, Japan). Anti-MT1-MMP, anti-MMP-2, anti-MMP-9, and tissue inhibitors of metalloproteinase (TIMP)-2 antibodies were gifted by Daiichi Fine Chemicals (Toyama, Japan). The following antibodies were used: anti-FLAG, anti-FN, and anti-tubulin antibodies (Sigma-Aldrich); anti-paxillin, anti-focal adhesion kinase (FAK), and anti-N-cadherin (BD Biosciences, Lexington, KY, USA); anti-phospho-Akt (Ser^{473}) and anti-Akt antibodies (Cell Signaling Technology, Danvers, MA, USA); anti-integrin β1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA); and anti-phosphorylated FAK ($pTyr^{397}$) antibody (BioSource, Camarillo, CA, USA). Hoechst 33342, rhodamine-labeled phalloidin, Alexa Fluor 680-conjugated wheat germ agglutinin (WGA), and Alexa Fluor-labeled secondary antibodies were purchased from Molecular Probes (Eugene, OR, USA).

2.4. Expression plasmids

FLAG epitope-tagged MT1-MMP (MT1F) and MT1-MMP fused with FLAG-tagged mCherry (MT1-mCherry) were constructed according to the methods described in a previous study [14].

2.5. Gelatin zymography

HT1080 cells were serum starved with 0.5% FBS/DMEM overnight and treated with the inhibitors in Opti-MEM for 24 h. The conditioned medium (CM) was analyzed by gelatin zymography, as previously described [14]. MMP-2 and MMP-9 levels in CM were measured by adding an equal volume of sample buffer. To detect cell-bound MMP-2 and MMP-9, cells were washed twice with phosphate-buffered saline (PBS) and dissolved in sample buffer using sonication. The samples were separated by electrophoresis on a SDS-polyacrylamide gel containing gelatin labeled with Alexa Fluor 680 (Molecular Probes). The gels were processed and monitored using an Odyssey infrared imaging system (LI-COR, Lincoln, NE, USA).

2.6. Western blotting

Cells were lysed in 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 5 mM EDTA, 2 mM Na_3VO_4, 2 mM NaF, 1% SDS, and a protease inhibitor cocktail (Nacalai Tesque, Kyoto, Japan). A bicinchoninic acid assay (Pierce, Rockford, IL, USA) was used to determine the protein concentration. The secreted proteins were concentrated by adding StrataClean Resin (Stratagene, La Jolla, CA, USA) to an appropriate volume of CM and were centrifuged for 2 h. After centrifugation, the supernatant was removed and a sample buffer was added to the precipitated resin. The samples were separated by electrophoresis on SDS-polyacrylamide gels and transferred to a nitrocellulose membrane.

2.7. Cell surface biotinylation

Cells were washed with ice-cold PBS and incubated with 0.5 mg/ml sulfo–NHS–biotin (Pierce) in PBS at 4 °C for 30 min. After washing, the cells were homogenized in a buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM Na_3VO_4, 1 mM NaF, 1% Triton X-100, and a protease inhibitor cocktail. The cell lysates were precipitated with Streptavidin Agarose Resin (Thermo Fisher Scientific, Rockford, IL, USA) after centrifugation. The precipitates were subjected to immunoblotting.

2.8. Pulldown of glycoproteins on concanavalin A beads

Cells were washed twice with ice-cold PBS and homogenized in a buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 2 mM $CaCl_2$, 2 mM $MnCl_2$, 1 mM EGTA, 1 mM PMSF, 1 mM Na_3VO_4, 1 mM NaF, 1% Triton X-100, and protease inhibitor cocktail. After centrifugation, the supernatants were used for pulldown with concanavalin A (Con A)-Sepharose beads (Sigma-Aldrich). The precipitates were subjected to immunoblotting.

2.9. Cell adhesion assay

Cell adhesion assay was performed as described previously [10]. In brief, 96-well plates were coated with 10 μg/ml of FN, 30 μg/ml of type I collagen, or 100 μg/ml of poly-$_L$-lysine (PLL) overnight at 4 °C and blocked with 1 mg/ml bovine serum albumin (BSA). HT1080 cells were serum starved with 0.5% FBS/DMEM overnight and treated with the inhibitors in Opti-MEM for 24 h. Cells were harvested as single cell suspensions and suspended for 30 min at 37 °C in Opti-MEM. The cells were allowed to adhere to the plate for 2 h at 37 °C and stained with 1% crystal violet. The crystal violet bound to the cells was eluted with 10% acetic acid and measured at an absorption of 590 nm.

2.10. Immunofluorescence staining

HT1080 cells were transfected with MT1F plasmid, serum starved, and treated with the inhibitors for 24 h. For cell surface staining, the cells were incubated for 30 min at 37 °C with anti-FLAG antibody. After washing, the cells were fixed with 4% paraformaldehyde for 15 min. Alternatively, the cells were fixed and permeabilized with 0.5% Triton X-100 and reacted with anti-FLAG antibody. The cells were visualized with Alexa™488-conjugated goat anti-mouse antibody, Hoechst 33342, and rhodamine-labeled phalloidin.

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2.11. FN-induced FAK and Akt activation

The cells were detached and kept in suspension in DMEM containing 2 mg/ml BSA for 30 min after treatment with 0.5 mg/ml trypsin inhibitor. Subsequently, they were replated on culture dishes coated with 10 μg/ml FN for 20 min. The cell lysates were analyzed by western blotting.

2.12. Wound healing assay

Serum-starved HT1080 cells were treated with the inhibitors in Opti-MEM for 24 h and allowed to adhere to FN-coated dishes for 2 h at 37 °C. Subsequently, confluent cell monolayers were scraped off. After adding Opti-MEM containing the inhibitors, wound closure was monitored for 15 h.

2.13. Cell invasion assay

Cell invasion was assayed using a modified Boyden chamber consisting Transwell membrane filters (Corning Costar, Cambridge, MA, USA). The upper surfaces of the membranes were coated with 1 mg/ml Matrigel matrix (BD Bioscience) and placed in 24-well tissue culture plates filled with 600 μl Opti-MEM with inhibitors. Serum-starved HT1080 cells (2×10^5 cells) were suspended in 100 μl Opti-MEM, added to each Transwell chamber, and cultured for 6 h as pretreatment. Subsequently, FN was added to the lower chambers (final concentration: 10 μg/ml). After further cultivation for 16 h, the membranes were fixed and stained. The number of crystal violet-stained cells on the lower surface was counted under a microscope.

3. Results

3.1. Design, synthesis, and COX-2 inhibitory activity of UTX-121

The design of novel analog was based on the structural modification of celecoxib as the lead compound by the bioisosterism principle. The sulfonamide of celecoxib and the carboxylic acid of compound **2** have a bioisosteric relationship, but carboxylic acid has high polarity and poor cell permeability. We, therefore, designed compound **3** (UTX-121) to protect compound **2** with methyl ester (Fig. 1A). In the synthesis process, a simple synthetic route was selected (Fig. 1A). The synthesized products were analyzed by ^{1}H NMR and MS. When the COX-2 inhibitory activity of celecoxib and UTX-121 was evaluated using the COX-2 (human) Inhibitor Screening Assay Kit as described in supplemental methods, the IC_{50} values were 0.02 μM and 237 μM for celecoxib and UTX-121, respectively.

3.2. UTX-121 suppressing MMP-9 activity and pro-MMP-2 activation

In order to study the effect of UTX-121 in tumor progression other than COX-2/PGE_2 pathway, HT1080 cells, which expressed little COX-2 at basal condition, were used in this study. The effect of UTX-121 on MMP activity was examined by gelatin zymography and western blotting. Serum-starved HT1080 cells were treated with either celecoxib or UTX-121 at 12.5 μM or 25 μM for 24 h, following which the conditioned media were analyzed. Treatment with 25 μM celecoxib slightly suppressed MMP-9 production and pro-MMP-2 activation (Fig. 1B). UTX-121 augmented this inhibitory effect of celecoxib on the activity of MMP-9 and MMP-2 since the inhibition of MMP-9 production and pro-MMP-2 activation was observed in cells treated with 12.5 μM UTX-121, which was enhanced by treatment with 25 μM UTX-121. MT1-MMP activates pro-MMP-2, and its enzymatic activity can be inhibited by TIMP-2, but not by TIMP-1 [10]. Celecoxib and UTX-121 treatment reduced the amount of secreted TIMP-2, suggesting that the suppression of MT1-MMP-mediated pro-MMP-2 activation by UTX-121 is not caused by TIMP-2 induction. To examine the effect of UTX-121 on ectopically expressed MT1-MMP, HT1080 cells stably expressing FLAG-mCherry-tagged MT1-MMP (HT1080-MT1mCherry) were established. When HT1080-MT1mCherry cells were exposed to the MMP inhibitor BB94, pro-MMP-2 activation was completely suppressed and MT1-MMP protein levels were increased due to inhibited autodegradation (Fig. 1C). UTX-121 treatment suppressed pro-MMP-2 activation in a dose-dependent manner; however, the ectopically expressed MT1-MMP levels remained unchanged, suggesting that UTX-121 suppresses MT1-MMP-mediated pro-MMP-2 activation without affecting MT1-MMP expression. Since pro-MMP-2 activation was executed by MT1-MMP expressed on the cell surface, the cell-bound MMP-2 activity was examined. Cell-bound MMP-2 activity and pro-MMP-2 activation were mostly inhibited in the cells treated with UTX-121 (Fig. 1D). These results indicate that UTX-121 may influence the level of cell surface MT1-MMP, which functions as a receptor for and activator of pro-MMP-2.

3.3. Effect of UTX-121 on MT1-MMP cell surface expression

Latent MT1-MMP (63 kDa) is activated in a post-Goldi compartment by proteolytic cleavage of the pro-peptide. The active 57-kDa MT1-MMP protein on the cell surface usually undergoes autocatalytic processing, yielding a membrane-tethering and a catalytic domain-lacking 44-kDa specie [15]. Western blotting analysis revealed that the 44-kDa degraded form of MT1-MMP was decreased by UTX-121 treatment in a dose-dependent manner, while both latent and active forms remained unchanged (Fig. 2A). We hypothesized that UTX-121 disrupts the cell surface trafficking of MT1-MMP. To examine this possibility, HT1080 cells were transiently transfected with FLAG-tagged MT1-MMP (MT1F), and cell surface MT1F levels were examined by immunofluorescence staining. Compared with DMSO treatment, Cell surface staining of MT1F in nonpermeabilized cells was significantly reduced by UTX-121 treatment and increased by BB94 treatment. When the cells were permeabilized, the level of MT1F signals were almost the same in DMSO- and UTX-121-treated cells (Fig. 2B). The reduction of cell surface MT1-MMP levels by UTX-121 was confirmed by pulldown of biotinylated proteins. Only biotinylated cell surface MT1-MMP, the active 57-kDa and degraded 45-kDa forms, were pulled down with streptavidin beads (Fig. 2C). When cells were treated with BB94, the active form of cell surface MT1-MMP was increased and the degraded form was not pulled down. UTX-121 treatment downregulated both forms of cell surface MT1-MMP. This was not only observed in the case of MT1-MMP but also with UTX-121 treatment, which reduced cell surface N-cadherin but not integrin β_1. These results suggest that UTX-121 treatment perturbs the trafficking of some cell surface proteins, including MT1-MMP.

3.4. Effect of UTX-121 on cell surface proteins

Nearly all proteins that are expressed in the plasma membrane or secreted are glycoproteins [16]. Glycosylation modifies the structures and functions of glycoproteins, and many MMPs contain *N*- or *O*-linked oligosaccharides [17]. Aberrant glycosylation is a common feature of malignant change and is suspected to contribute to the tumor progression. COX-2 has been implicated in aberrant glycosylation in tumor cells [2]. To examine the effect of UTX-121 on glycosylation, Con A precipitation assay was performed. Treatment of cells with *N*-linked glycosylation inhibitor tunicamycin significantly decreased the amount of N-cadherin and

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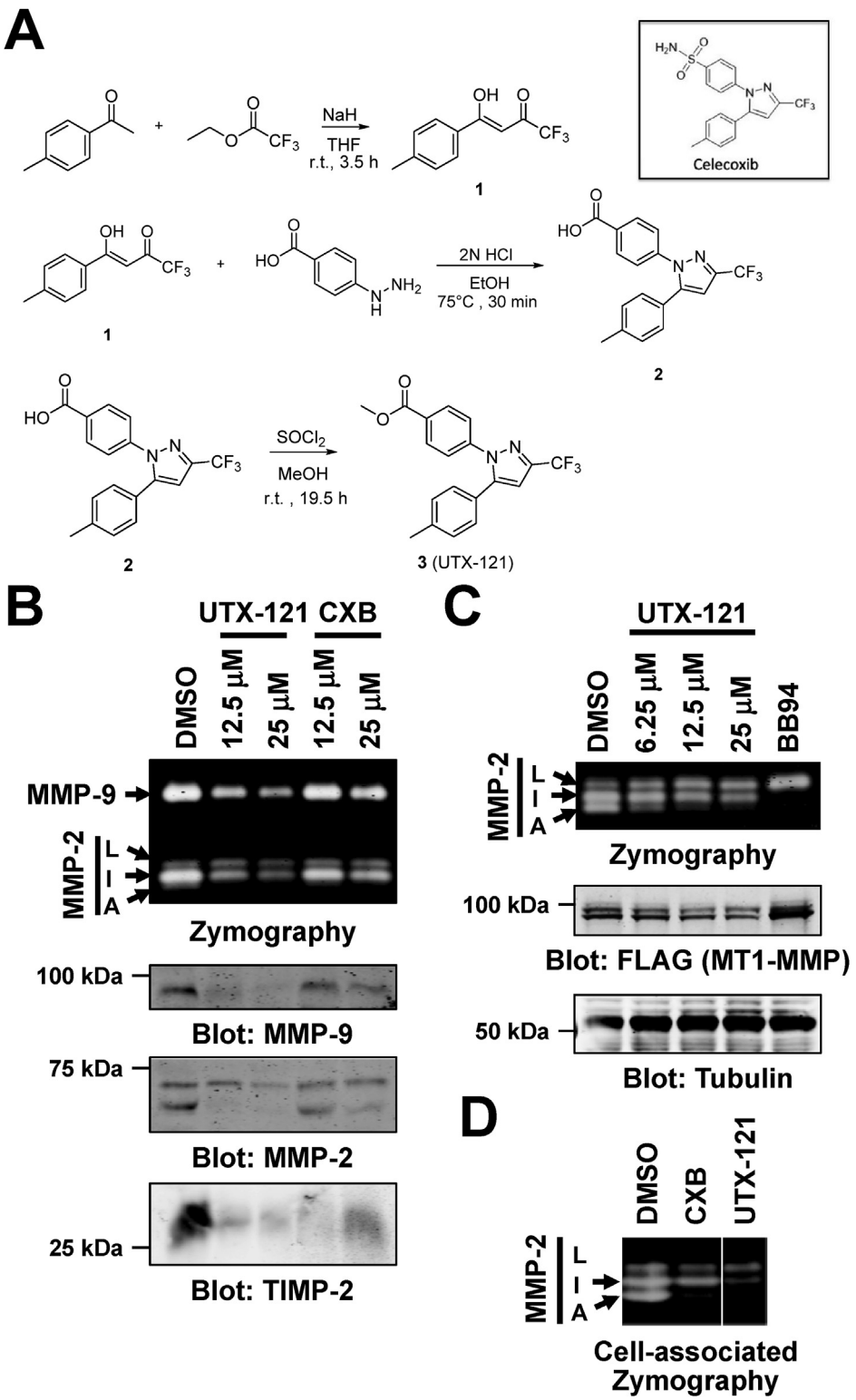

Fig. 1. UTX-121 inhibits pro-MMP-2 activation. (A) Design and synthesis of UTX-121. (B) Serum-starved HT1080 cells were treated with either UTX-121 or celecoxib (CXB) at indicated concentrations for 24 h. The conditioned media (CM) were analyzed by gelatin zymography and immunoblotted with anti-MMP-9, anti-MMP-2, and anti-TIMP-2 antibodies. L, latent form; I, intermediate form; A, active form of MMP-2. (C) Serum-starved HT1080-MT1mCherry cells were treated with either UTX-121 or BB94 (2 μM) for 24 h. CM were analyzed by gelatin zymography. The cell lysates were immunoblotted with anti-FLAG and anti-tubulin antibodies. (D) Serum-starved HT1080 cells were treated with indicated inhibitors (25 μM) for 24 h. The cells were homogenized and analyzed by gelatin zymography.

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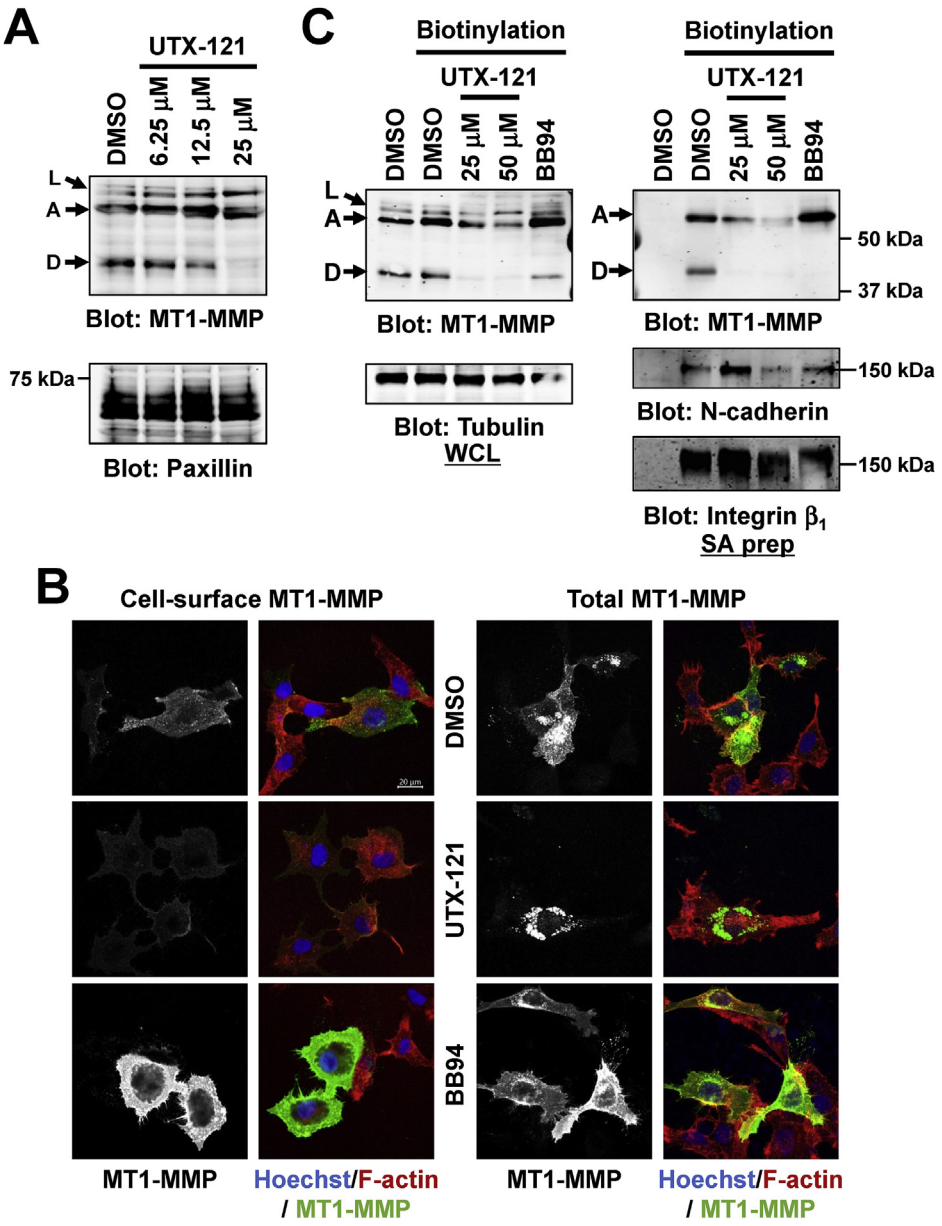

Fig. 2. UTX-121 suppresses cell surface expression of MT1-MMP. (A) HT1080 cells were treated with UTX-121 at indicated concentrations for 24 h. The cell lysates were immunoblotted with anti-MT1-MMP and anti-paxillin antibodies. L, latent form; A, active form; D, degraded form of MT1-MMP. (B) HT1080 cells were transfected with MT1F. The cells were treated with either UTX-121 (25 μM) or BB94 (2 μM) for 12 h and incubated with anti-FLAG antibody for 30 min at 37 °C. After washing, immunofluorescence staining was performed under either permeabilized (total) or nonpermeabilized (cell surface) conditions. Bar, 20 μm. (C) Cell surface biotinylation and immunoprecipitation. The whole-cell lysates were immunoblotted with anti-MT1-MMP and anti-tubulin antibodies. Biotinylated cell surface proteins were precipitated with streptavidin agarose. The precipitates (SA prep) were immunoblotted with anti-MT1-MMP, anti-N-cadherin, and anti-integrin β_1 antibodies.

integrin β_1, which were precipitated with Con A-Sepharose beads (Fig. 3A). This decrease was also observed in cells treated with UTX-121. WGA binding revealed that UTX-121 reduces *N*-linked glycosylation in Con A-precipitates similar to tunicamycin. MT-MMP is known to be *O*-glycosylated [17]. Latent and active form of MT1-MMP were precipitated with Con A-Sepharose beads, which were not affected by UTX-121 treatment. Tunicamycin treatment resulted in the suppression of pro-MMP-2 activation, autodegradation of MT1-MMP, and accumulation of latent MT1-MMP.

As glycosylation of integrins affects cell adhesion and migration [18], the effect of UTX-121 on cell adhesion and spreading was examined. Treatment of cells with UTX-121 impaired the adhesion of cells to FN but not to PLL and type I collagen (Fig. 3B). Thus, cell spreading on FN was impeded in the UTX-121-treated cells compared with DMSO-treated cells (Fig. 3C). FAK is a widely expressed nonreceptor protein tyrosine kinase localized in focal adhesions. FAK becomes activated and phosphorylates tyrosine in response to adhesion of cells to ECM. Activated FAK undergoes autophosphorylation at Tyr-397 and, thereby, associates a number of signaling molecules and structural proteins. PI3K is one of the FAK-activated proteins, which recruits and activates its downstream target Akt [19]. The PI3K/Akt pathway is the most frequently activated in human cancer tissues and plays a pivotal role in proliferation, survival, and metastasis of cancer cells. The effect of UTX-121 on FN-induced FAK and Akt activation was examined. HT1080 cells treated with inhibitors were either held in suspension or

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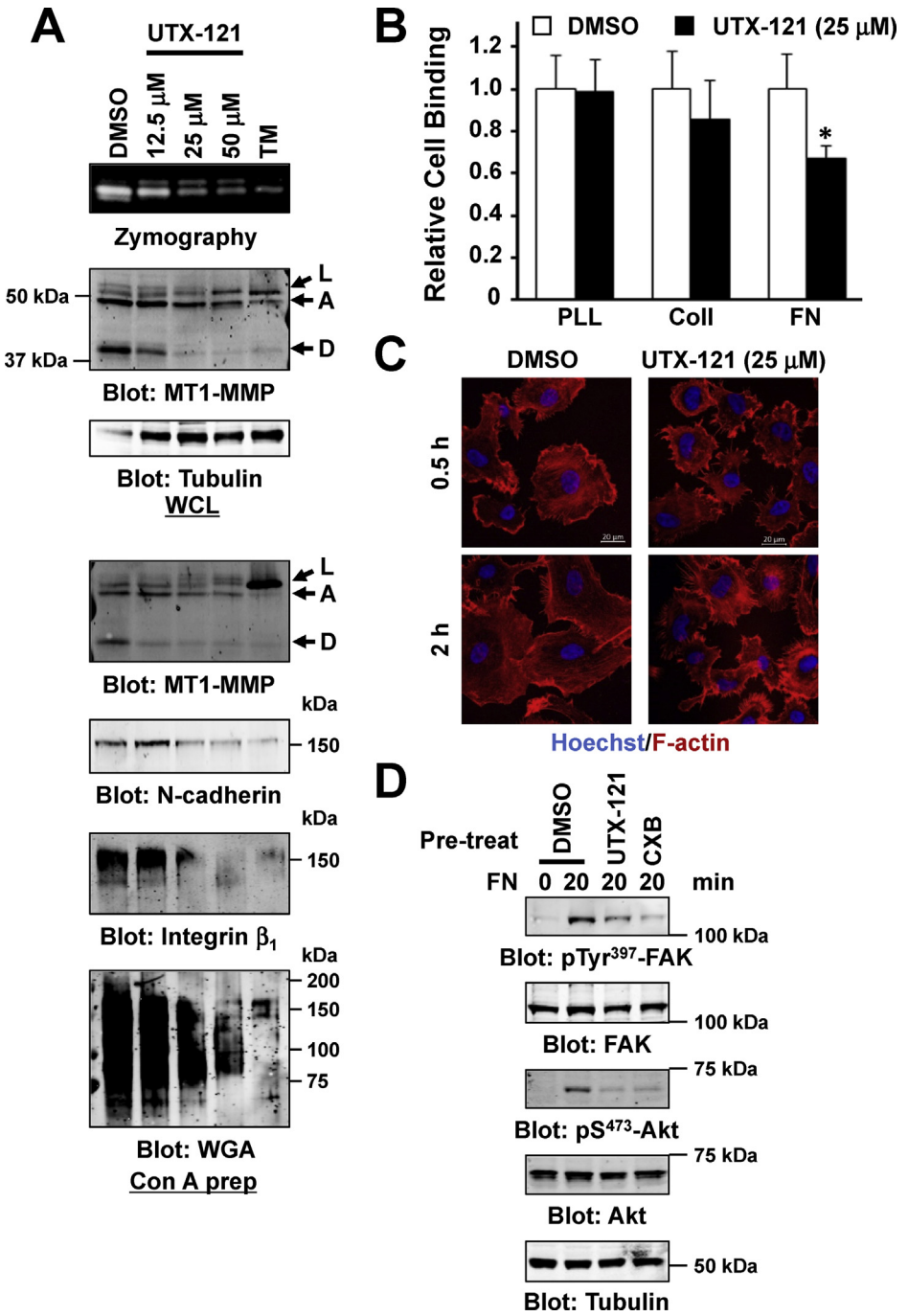

Fig. 3. UTX-121 reduces N-glycosylation. (A) HT1080 cells were treated with either UTX-121 or tunicamycin (TM: 25 μM) for 24 h. The conditioned media (CM) were analyzed by gelatin zymography. Cell lysates were immunoblotted with anti-MT1-MMP and anti-tubulin antibodies. Alternatively, glycoproteins were pulled down with Con A-Sepharose beads. The precipitates (Con A prep) were immunoblotted with anti-MT1-MMP, anti-N-cadherin, and anti-integrin β_1 antibodies, and wheat germ agglutinin (WGA). (B) Cell adhesion assay. Error bars, S.D.; *, $P < 0.01$ versus DMSO; PLL, poly-L-lysine; Coll, type I collagen; FN, fibronectin. (C) HT1080 cells were treated with UTX-121 (25 μM) for 24 h. The cells were detached, kept in suspension, and replated on FN-coated cover slips for 30 min or 2 h. Cells were stained with Hoechst 33342 (Hoechst) and rhodamine-labeled phalloidin (F-actin). Bar, 20 μm. (D) HT1080 cells were treated with either UTX-121 (25 μM) or CXB (25 μM) for 24 h. The cells were detached, kept in suspension, and replated on FN-coated dishes for 20 min. Cell lysates were immunoblotted with anti-phospho-FAK (Blot: pTyr^{397}-FAK), anti-FAK, anti-phospho-Akt (Blot: pS^{473}-Akt), anti-Akt, and anti-tubulin antibodies.

attached to FN. When the cells were treated with DMSO, FAK autophosphorylation at Tyr-397 was effectively induced in the cells stimulated with FN, but not in the suspended cells (Fig. 3D). This FN-induced FAK phosphorylation was attenuated by either UTX-121 or celecoxib treatment, which caused the reduction of Akt activation. These results suggest that glycosylation of cell surface proteins is impeded by celecoxib and its derivative UTX-121, which results in the suppression of cell attachment to FN, FAK autophosphorylation, and Akt activation.

3.5. Effect of UTX-121 on cell invasion

The FAK/Akt signaling pathway plays a crucial role in cell migration and invasion [19]. The effect of UTX-121 on HT1080 cell

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migration and invasion was tested. As expected, wound closure assay revealed that FN-stimulated cell migration was suppressed by UTX-121 treatment (Fig. 4A). FN-stimulated invasion of HT1080 cells through Matrigel matrix was reduced to 57.9% and 29.9% by celecoxib treatment and UTX-121 treatment, respectively (Fig. 4B). These results strongly suggest that downregulation of glycosylation in cell adhesion molecules and inhibition of MT1-MMP activity by UTX-121 treatment impede the invasion of HT1080 cells.

4. Discussion

In this study, we designed and synthesized UTX-121 to augment the COX-2-independent pharmacologic activities of celecoxib. UTX-121 treatment suppressed MMP-9 production, MT1-MMP-mediated MMP-2 activation, cell adhesion, and cell migration, resulting in the inhibition of HT1080 cell invasion into the Matrigel.

MMP-2 and MMP-9 are type IV collagenase/gelatinase, and their expression is often associated with tumor malignancy [20]. Celecoxib suppressed MMP-9 production by inhibiting nuclear factor-κB and activator protein 1 [12,13,21]. UTX-121 was more effective in reducing the MMP-9 production in HT1080 cells than celecoxib (Fig. 1). Activation of pro-MMP-2 by MT1-MMP contributes to the malignant conversion of various tumors [9–11]. We found here that UTX-121 suppresses pro-MMP-2 activation by disrupting cell surface expression of MT1-MMP (Fig. 2). Aberrant glycosylation occurring in cancer cells alters cell adhesion, molecular trafficking, and signal transduction, and thus appears to induce tumor cell invasion and metastasis [16–18]. COX-2/PGE_2 pathway induced sialyltransferase-3 expression in MDA-MB-231 breast cancer cells, which was suppressed by celecoxib, suggesting that COX-2 might affect the glycosylation of tumor cells [2]. Similarly, UTX-121 and tunicamycin attenuated *N*-linked glycosylation in HT1080 cells (Fig. 3). MT1-MMP trafficking inhibition in the cell surface by UTX-121 can be possibly attributed to the perturbation of *N*-linked glycosylation, which may affect the cell surface trafficking of MT1-MMP. This may be supported by our results, which show that tunicamycin inhibits MT1-MMP-mediated pro-MMP-2 activation, and the fact that tunicamycin suppresses proliferation and migration of hepatocellular carcinoma cells through CD44 and MAPK pathways [22], which regulates MT1-MMP activity.

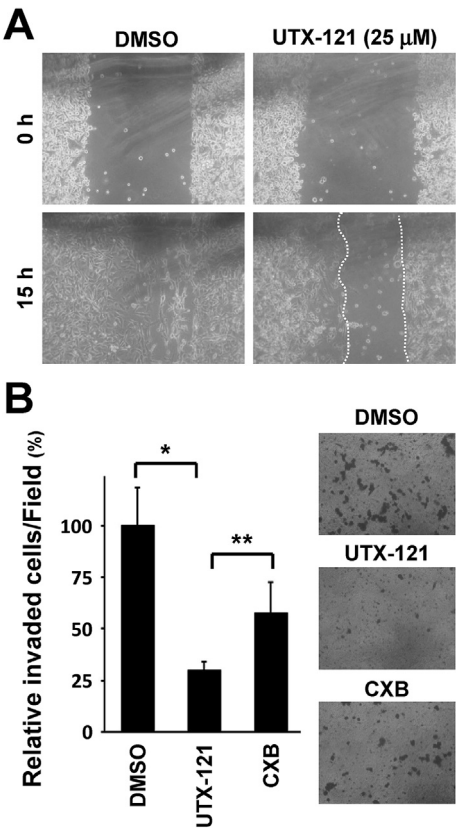

Fig. 4. UTX-121 inhibits invasion. (A) Wound closure assay on FN. Dashed lines indicate unclosed wound area. (B) Cell invasion assay. Error bars, S.D.; *, $P < 0.01$; **, $P < 0.05$.

Tumor cell invasion is a complex process that includes cell adhesion to ECM, ECM degradation, and cell migration [23]. Cell adhesion to ECM governs cellular functions, including gene expression, proliferation, survival, and motility. The engagement of integrins by attachment of cells to ECM stimulates the activation of FAK, which functions as a scaffold to activate various signals [19]. When macrophages were attached to rat aortic smooth muscle cell-derived ECM, COX-2 and MMP-9 expressions was induced, which was inhibited by celecoxib [13]. When HT1080 cells were treated with UTX-121, cell adhesion to FN was reduced, which resulted in the suppression of cell spreading, migration, and FAK/Akt activation (Fig. 3). This is probably due to the inhibition of integrin β_1 glycosylation by UTX-121 since the cellular receptors for FN are integrins $\alpha_5\beta_1$, $\alpha_9\beta_1$, and $\alpha_4\beta_1$ and α_v-integrins. Together with MMP inhibition, UTX-121 significantly decreased FN-stimulated migration and invasion of HT1080 cells (Fig. 4). As UTX-121 had little inhibitory effect on COX-2, its anti-tumor activities seemed to underlie a COX-2-independent mechanism. However, we cannot rule out the effect of UTX-121 on COX-1 and COX-3 [24]. Hence, further studies are required to examine the molecular mechanism by which UTX-121 perturb the trafficking of membrane proteins to the cell surface.

In conclusion, we succeeded in the development of the celecoxib derivative UTX-121 possessing the inhibitory activities of MMPs, cell migration, and cell invasion. UTX-121 may be a potent lead compound that can be used to develop a novel anti-tumor drug, which improves the COX-2-independent pharmacologic activities of celecoxib.

Declaration of competing interest

None.

Acknowledgement

This work was supported partly by Extramural Collaborative Research Grant of Cancer Research Institute, Kanazawa University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2019.10.092.

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Please cite this article as: H. Yamahana et al., A novel celecoxib analog UTX-121 inhibits HT1080 cell invasion by modulating membrane-type 1 matrix metalloproteinase, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2019.10.092