Recombinantly expressed MeICT, a new toxin from *Mesobuthus eupeus* scorpion, inhibits glioma cell proliferation and downregulates Annexin A2 and FOXM1 genes

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Abstract Gliomas are highly invasive and lethal malignancy that do not respond to current therapeutic approaches. Novel therapeutic agents are required to target molecular mechanisms involved in glioma progression. MeICT is a new short-chain toxin isolated from *Mesobuthus eupeus* scorpion venom. This toxin contained 34 amino acid residues and belongs to chloride channels toxins. In this study, the coding sequence of MeICT was cloned into the pET32Rh vector and a high yield of soluble recombinant MeICT was expressed and purified. Recombinant MeICT-His significantly inhibited the proliferation and migration of glioma cells at low concentration. In vivo studies showed that MeICT was not toxic when administered to mice at high doses. We also determined the effect of MeICT on the mRNA expression of MMP-2, Annexin A2 and FOXM-2 that are key molecules in the progression and invasion of glioma. Expression of Annexin A2 and FOXM1 mRNA was significantly down-regulated following treatment with MeICT. However, no significant decrease in the expression of MMP-2 gene was identified. In this study a short toxin with four disulfide bonds was successfully produced and its anti-cancer effects was detected. Our findings suggest that recombinant MeICT can be considered as a new potent agent for glioma targeting.

Keywords Recombinant MeICT peptide · Glioma cells · Annexin A2 · FOXM1 · MMP-2

Introduction

Malignant glioma is a common primary brain tumor and is considered among the most lethal cancers (Liang et al. 2020). The tumor is highly invasive with fast cell proliferation and unusual tendency to spread to healthy brain tissue. Glioma cells spread through two main mechanisms: degradation of the extracellular matrix (ECM) by matrix metalloproteinases (MMPs) such as MMP-2 and deformation of the cell shape via ionic gradients that occurred by overexpression of ion channels such as chloride (Cl⁻) channels (Turner and Sontheimer 2014; Dardevet et al. 2015). The search for therapeutic agents that target these molecular mechanisms without affecting normal tissues is increasingly being considered.

MeICT, a novel short-chain toxin, was isolated from Iranian *Mesobuthus eupeus* scorpion in our laboratory (Ilkhanizade et al. 2011). MeICT contains 34 amino acids crosslinked by four disulfide bridges and displays 81% sequence similarity with chlorotoxin (CTX). CTX from the venom of *Leiurus quinquestriatus* scorpion contains 36 amino acids and a compact structure of an α–helix and three β-sheets. This peptide selectively binds to chloride channels in glioma cells and inhibits their migration and invasion (Dardevet et al. 2015). CTX can also interact with MMP-2.
and Annexin A2 (ANXA2) that both are involved in invasion of glioma cells (Deshane et al. 2003; Kesavan et al. 2009). CTX and its analogs have received special attention for gliomas targeting (Cheng et al. 2014; Xu et al. 2016). In addition, CTX like peptides such as BmKCT and AaCTX isolated, respectively, from Buthus martini’s Karsch and Androctonus australis scorpion venoms show anti-glioma effects (Fau et al. 2007; Rjeibi et al. 2011). In silico studies on MeICT confirmed its interaction with Cl− channel and MMP-2 protein (Farsani et al. 2015; data are publishing).

Most peptide toxins are produced by purification from the animal venoms or chemical synthesis that both are high-cost procedures. Recombinant production of toxins in Escherichia coli is more economical and easier method. However, the small size of peptide toxins and high number of disulfide bonds cause low yield of recombinant toxins (Lyukmanova et al., 2010). There are some fusion tags that are able to enhance the solubility of peptide toxins and protect them from degradation (Esposito et al. 2006; Bogomolovas et al. 2009). In this study, we tried to produce recombinant MeICT in E. coli expression system by using a new designed vector based on pET vectors. The pET32Rh vector was previously constructed in our laboratory to purify recombinant proteins easier and more cost-effective (Rezaei et al. 2021). To determine the anti-cancer function of recombinant MeICT, we investigated its effect on the proliferation and migration of glioma cells. To elucidate the molecular mechanisms involved in MeICT inhibitory effects, mRNA expression of MMP-2, ANXA2 and FOXM1 which are critically associated with glioma progression were investigated following rMeICT treatment.

MMP-2 overexpression is related to glioma malignancy and promotes glioma cell migration through degradation of ECM. Inhibition of MMP-2 expression significantly attenuates cell invasion (Roomi et al. 2007). Overexpression of ANX A2 has also been observed in glioma cells while it does not express in healthy brain tissue. ANXA2 expresses on surface of the cancer cells and upregulates ECM degradation via activation of pro-MMPs (Sharma 2019). ANX A2 as a tumor-associated protein promotes cancer proliferation, migration, invasion, angiogenesis, inhibition of apoptosis and therapeutic resistance. The role of ANX A2 in cancer development makes this protein an interesting molecule for targeted therapy (Zhai et al. 2011).

FOXM1 belongs to the FOX (Forkhead Box) transcription factor family and is a key transcriptional regulator of the cell cycle. This factor is significantly involved in the tumorigenesis process of glioma (Cheng et al. 2013). Overexpression of FOXM1 is critical in many cancers and promotes proliferation, migration and invasion of glioma cells. Interestingly, FOXM1 has a binding site in the promoter of the MMP-2 gene and regulates its expression (Dai et al. 2007). Elevated FOXM1 level enhances glioma grade and invasion, whereas targeting of FOXM1 significantly suppresses the invasion of glioma cells (Zhang et al. 2017).

This study indicated that recombinant MeICT suppressed growth and migration of glioma cells and decreased the mRNA expression of ANX A2 and FOXM1 with no toxic effect on living organisms. These results suggest that MeICT can be considered as a new potential targeting agent for glioma therapy.

**Materials and methods**

**Animals and cells**

The U87-MG, human glioblastoma astrocytoma cell line, was kindly provided by Dr. Hossein Asadi from Mahan Research Institute, Kerman. Cells were grown in complete DMEM medium supplemented with 10% fetal bovine serum (FBS) and maintained in a humidified incubator with 5% CO₂ at 37 °C. The male BALB/c mice, weighing 20 to 30 g, were purchased from Razi Animal Center, Tehran. The experimental animal procedures were performed in accordance with the National Institutes of Health guide for the care and use of Laboratory animals and by the Animal Ethics Committee of Shahrekord University (IR. SKU.REC.1399.017).

**Bioinformatics studies**

The alignment of sequences was performed by ClustalW2 server (http://www.ebi.ac.uk/tools/msa/clustalw2/). DbD2 server (http://cptweb.cpt.wayne.edu/DbD2) was used to predict disulfide bond formation. Sequence homology analysis was performed by the NCBI BLAST server (http://www.ncbi.nlm.nih.gov/blast).
The tertiary structure of MeICT was generated through Homology and Basic modeling by Modeler software. PDB structure of Insectotoxin I5a peptide with similar sequence to MeICT were selected as the template. The top 10 models of MeICT in PDB format were selected and the structure with the lowest molpdf energy was considered as the best proposed structure. Finally, it was examined by Cn3D software.

Cloning and expression of MeICT

The pET32Rh vector was used to clone of MeICT gene. This vector is a modified version of the pET32b plasmid that has lost His-tag sequence upstream of the multiple cloning sites. Therefore, the peptide containing C-terminal His-tag can be separated from thioredoxin part by two consecutive Ni–NTA purifications (Rezaei et al. 2021). The MeICT-encoding sequence and pET32Rh vector were digested by restriction endonucleases, BamHI and XhoI, and ligated with T4 ligase (Takara, Japan). The recombinant pET32Rh-MeICT vector was transformed into competent E. coli BL21 bacteria. Transformed bacteria were cultured in liquid LB medium up to OD600 = 0.6 and induced by 0.5 mM IPTG for 7 h at 28 °C. The bacteria were precipitated at 4000 rpm for 15 min, 4 °C and then 100 µl of lysis buffer (10 mM Tris–HCl pH = 8, 10 mM EDTA) was added and gently mixed to homogeneous suspension. Lysozyme solution at a concentration of 1 mg/ml was added and placed on ice for 20 min, the lysate was centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatant was analyzed on 12% SDS-PAGE and stained with Coomassie blue.

Purification of recombinant rMeICT

About 250 ml media containing recombinant bacteria was cultured and induced for 7 h at 28 °C. Harvested cells were suspended in 4 ml of lysis buffer containing 1 mM imidazole, then sonicated and incubated on ice for 20 min. The lysate was centrifuged and the supernatant was mixed with 1 ml of Ni–NTA resin (Qiagen, Germany) and gently shooked for 1 h on ice. The mixture was loaded on an affinity column, washed with washing buffer (50 mM NaH2PO4, 300 mM NaCl and 20 mM imidazole) and eluted two times with elution buffers containing 50 mM NaH2PO4, 300 mM NaCl, 250 mM and 500 mM imidazole, respectively. The eluted fraction was dialyzed against PBS solution overnight. Dialyzed samples containing recombinant Thioredoxin-MeICT (Trx-MeICT) were cleaved by 0.1 mg/ml enterokinase enzyme (Sigma, Germany) and analyzed on 12% Tris-Tricine-SDS-PAGE. In order to purify rMeICT peptide from thioredoxin part, the peptide was re-purified by Ni–NTA column and dialyzed again. The rMeICT concentration was determined by Bradford assay.

Glioma cell viability assay

The growth and survival of glioma cells were measured using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay. The cells were seeded at 5×10^3 density in 96-well plate wells. The plate was incubated for 24 h and then treated with rMeICT concentrations of 1, 2, 3, 4, 5, and 6 µM in triplicate. After 24 h, 20 µl solution of 5 mg/ml MTT (Sigma, Germany) in PBS was added and incubated at 37 °C for 4 h. The medium was discarded and 150 µl of MTT solvent (containing dimethyl sulfoxide) was added to each well, plate was incubated for 30 min. in the dark to dissolve formazan crystals. The absorbance was measured at 570 nm. The percentage of living cells was calculated using: optical density (OD) value of treated cells/ OD value of untreated cells × 100.

Wound healing assay

Cells (2×10^5) were seeded into a 12-well plate until reaching confluen of 80–90%, then were gently scratched using a 100 µl pipette tip and washed carefully with PBS. New medium was added to the wells and cells were treated with 3 and 5 µM concentration of rMeICT. Images were captured at 0, 12 and 24 h and data were analyzed using ImageJ software (NIH, USA). Wound closure rate was calculated by placing the initial and final wound distance in the following equation: 100% × [(initial−final)/initial].

RNA extraction, cDNA synthesis and quantitative real time PCR

Cells were treated with 5 µM rMeICT and harvested after 24 h. Total RNA was extracted by TRIzol Reagent (Fermentas, USA). The cDNA was constructed using Prime Script RT Kit (Takara, Japan) according
to the manufacturer’s instructions. Quantitative real-time PCR with SYBR green method was used to evaluate the quantitative expression of ANXA2, FOXM1, and MMP-2 genes. The PCR was performed using following primers, ANXA2 forward: 5′CTGTGTGGTG GGAGATGACTG3′, ANEXA2 reverse: 5′CGGGGACTGTATTCCGCAAG3′; FOXM1 forward: 5′CAGTGGCTCGAAAGATGAG3′, FOXM1 reverse: 5′TCCTCAACCTTAACCTGTGC3′; MMP-2 forward: 5′CAGTGCTTCGAAAGATGAG3′, MMP-2 reverse: 5′TCCTCAACCTTAACCTGTGC3′. PCR condition consisted of 94 °C for 20″ followed by 40 cycles of 94 °C for 3″ and 60 °C for 1 min. Data were normalized to β-actin gene and relative quantities were calculated using $2^{-\Delta\Delta C_{t}}$ equation.

**In vivo toxicity analysis**

The in vivo toxicity of rMeICT was evaluated in mice by intraperitoneal injection. Different doses of toxin were injected in a range of 0.5 µg to the increasing amount of 1, 3, 6, and 10 µg per 20 g body weight of mice. Five mice were used for each dose. Control mice were injected with PBS containing 0.1%(w/v) BSA solution. Animals were kept in cages under standard laboratory conditions with 12:12-h light–dark cycles. The mice were observed regularly for any clinical signs of toxicity, physical and behavioral changes up to 72 h post-injection.

**Statistical analysis**

Data were analyzed using SPSS software. Student’s t-test and one-way ANOVA were used to analyze the variances between two groups and more, respectively. A value of $P<0.05$ was considered statistically significant. Each experiment was repeated three times.

**Results**

**MeICT structure prediction**

Based on sequence homology, MeICT peptide was classified as a CTX-like peptide that can affect chloride channel (Fig. 1A). The similarity of MeICT peptide with CTX, BmKCT and AaCTX was determined 81%, 82% and 68%, respectively. Eight conserved cysteines in MeICT peptide form four disulfide bonds C1-C4, C2-C6, C3-C7 and C5-C8 that is consist with conventional pattern of Cl− channel toxins (Fig. 1B). The tertiary structure of MeICT was obtained by homology modeling using structure of Insectotoxin I5A templates. MeICT structure comprises an alpha helix formed by residues 11 to 20 and three β-sheets that one of them is located in the N-terminal and two of them are near the carboxyl terminal. The positions of the alpha helix and β-sheets are similar to CTX secondary structure (Fig. 1C). However, because of the fewer number of residues; the length of MeICT β-strands is shorter than CTX.

**Expression and purification of MeICT peptide**

The gene encoding MeICT toxin was cloned into pET32Rh expression vector. The schematic structure of the recombinant MeICT fused to thioredoxin (Trx) with an enterokinase cleavage site in the N-terminal and a His-tag in the C-terminal has been shown in Fig. 2A. Recombinant Trx-MeICT with an expected molecular weight around 25 kDa was observed in the soluble phase in SDS-PAGE (Fig. 2B). The rTrx-MeICT was purified by Ni–NTA affinity chromatography (Fig. 2C) and cleaved using enterokinase enzyme. MeICT-His peptide was re-purified and detected around 4.5 kDa on Tris-Tricine-SDS-PAGE (Fig. 2D). The amount of rMeICT-His peptide was determined about 0.6 µg/µl with Bradford assay.

**Cytotoxicity assay on glioma cells**

The effect of rMeICT on glioma cell proliferation and survival was analyzed using MTT test. The viability of cells was investigated in peptide concentrations of 1 to 6 µM. The results showed that rMeICT inhibits cell proliferation in a dose-dependent manner. The peptide concentration in which 50% of the cells are killed (IC50) was determined approximately 3.8 µM (Fig. 3A).

**Wound healing assay**

The effect of rMeICT on the migration of glioma cells was assessed by 3 and 5 µM of the peptide. The ability of cells to fill the distance between cell boundaries depended on the dose of rMeICT.
Compared with the control that was completely filled after 24 h, the percentage of wound closure was determined about 58% and 22% when treated with 3 and 5 μM of rMeICT, respectively (Fig. 3C).

Evaluation of ANXA2, MMP-2 and FOXM1 gene expression

The effect of rMeICT on expression of ANXA2, FOXM1 and MMP-2 genes in glioma cells was investigated by quantitative real-time PCR. Due to the significant role of these genes on cell migration, cells were treated with 5 μM of rMeICT for 24 that was the concentration with high inhibition effect on migration. The mRNA expression levels of FOXM1 and ANXA2 were significantly decreased compared with the control by 77% and 98.6%, respectively ($P < 0.01$) (Fig. 4). Despite the decrease in mRNA expression level of MMP-2, no statistically significant difference was determined.

In vivo toxicity

The safety of rMeICT was evaluated by intraperitoneal injection of five doses of 0.5 to 10 μg peptide in mice. The rMeICT did not induce death or any symptom after 72 h and the following days. The general symptoms of venom injection in animals were not seen in treated mice such as pain, muscle contractions, tissue paralysis, eye and nose bleeding. The experience of pain occurs due to tissue damage and is started licking the injection site, muscle spasm and twitching that did not appear in mice. In addition, other uncommon toxin symptoms were not detected such as ecchymosis, vomiting, profuse sweating, ataxia and behavior alterations (restlessness, somnolence and irritability). Therefore, it seems that this peptide is not toxic on mammals because the amount of 10 μg/20 g body weight is considered a high dose.

Discussion

Malignant glioma is the most aggressive brain tumor and does not respond to common therapeutic
approaches. Increased attention has focused on CTX-like peptides and their analogs for targeting glioma cells (Cheng et al. 2014; Xu et al. 2016). Identification of new CTX-like peptides can introduce more efficient therapeutic agents and elucidate the molecular mechanisms of their function. In this study, recombinant MeICT, was produced by cloning into pET32Rh vector. This vector facilitates the production of peptide using a two-step purification process and high-cost techniques such as HPLC (High-performance liquid chromatography) do not need to the purification of recombinant protein (Rezaei, et al. 2021). Recombinant production of toxins is a challenging task due to the small size of toxins that results in peptide distraction in expression host and also their cysteine-rich structures that lead to protein aggregation. Although MeICT contains four disulfide bonds, its fusion with thioredoxin resulted in high expression level of the soluble peptide. The presence of thioredoxin in N-terminal of MeICT not only protects the peptide from degradation in E. coli, but also increases solubility of the protein by correct disulfide bond formation (LaVallie et al. 2000). In previous study on screening 13 different fusion partner for toxin expression in E. coli, the fusion to thioredoxin gave the highest yield of soluble toxin (Bogomolovas et al. 2009). In this study we also obtained high yield of purified MeICT after two step purification. The anti-glioma activity of rMeICT-His demonstrated that His-tag in the C-terminal of peptide did not affect its function. It was consistent with a previous study that showed activation of recombinant His-CTX, although His-tag had been located in the N-ter of CTX (Deshane et al. 2003). It could be for the compact and specific structure of these peptides that is not affected by His-tag in C- or N-terminals.

In vivo toxicity of MeICT peptide was evaluated by peritoneal injection in mice. Due to the large number of blood vessels in this area, the peptide is rapidly introduced into the bloodstream. The signs of toxin injection are classified to nonneurological signs such as salivation, urination, diarrhea,
dysphagia, muscle spasm, paralysis, hyperthermia and neurological symptoms such as eye and nose bleeding, lymphangitis, necrosis and ulcerate. The high amount of MeICT did not induce any neurological and nonneurological symptoms in mice that suggests this toxin can be an insect-specific peptide and does not affect normal mammalian cells.

The amino acid sequence of MeICT showed obvious homology to toxins specific for chloride channels. The interaction of MeICT with Cl⁻ channel was determined by in silico studies (data are publishing).
Our results demonstrated that MeICT inhibits glioma cell proliferation and migration in a dose-dependent manner. Previous studies have shown that CTX, BmKCT and AaCTX suppress growth and migration of glioma cells with different concentrations (Fau et al. 2007; Rjeibi et al. 2011; Xu et al. 2016). These differences might be related to how they interact with their targets. Although these toxins belong to the family of chloride channel toxins, they might also interact with other targets such as MMP-2 and ANXA2 (Deshane et al. 2003; Kesavan et al. 2009; Fu et al. 2011; Othman et al. 2017). It seems that the invasion of glioma cells is correlated with MMP-2 expression and secretion. Fu et al. showed that the alpha helix region of BmKCT interacts with the catalytic domain of MMP-2 and inhibits its function via R14, R17 and K15 residues (Fu et al. 2011). The interaction of MeICT with MMP-2 was investigated by molecular docking in our previous study and it was determined that the alpha helix region of MeICT peptide interacts with the catalytic domain of MMP-2 (Farsani et al. 2015). Comparison of the alpha helix region of CTX, BmKCT, MeICT and AaCTX showed that this region is relatively conserved in MeICT, CTX and BmKCT (Fig. 1A). Substitutions of residues with similar properties have occurred in this region, for example R14 in CTX and BmKCT peptides has been replaced by the K14 in MeICT that both are positively charged residues. In addition, D18 in CTX and MeICT has been replaced by E18 in BmKCT that both are negatively charged amino acids. This region is more distinctive in AaCTX, for example an uncharged residue, alanine, has been replaced in both positions 14 and 18. It has been suggested that the lower activity of AaCtx on migration of glioma cells is attributed to increasing the net charge of AaCTX to +4, as well as the absence of negatively charged residues in alpha helix and N-terminal loop (Rjeibi et al. 2011).

In MeICT peptide, the positive net charge has been determined +2 and the negatively charged amino acids D18 and D9 are present in the alpha-helix and N-ter respectively. On the other hand, Othman et al. showed that residues 29–36 (8 C-ter residues) of CTX directly interact with MMP-2 (Othman et al. 2017). Furthermore, a recent study indicated that eight C-terminal residues of CTX are fundamental for its bioactivity and inhibition of cell migration (Dastpmam et al. 2019). Seven C-ter residues of CTX and MeICT are identical and only Y29 in CTX has been replaced with F29 in MeICT, that both are aromatic residues with similar biochemical properties. Overall, MeICT displays more similarity with CTX than with BMKCT and AaCTX. The amino acid similarity of MeICT, AaCTX and BmKCT with CTX was determined 81%, 61% and 76%, respectively. Therefore, it is possible that MeICT interacts with chloride channel and MMP-2 and inhibits progression of glioma cells, in similar ways like CTX.

Although the effective role of CTX and BmKCT in targeting glioma has been identified, the molecular mechanisms involved in this process have not been well described yet. In this study, we investigated the mRNA expression levels of ANXA2, MMP-2 and FOXM1 genes, which all are up-regulated in glioma cells and contribute to glioma migration and invasion. Previous studies have been shown that CTX and BMKCT significantly reduce MMP-2 protein secretion in glioma cells; however, no differences in MMP-2 mRNA expression were determined (El-Ghlban et al. 2014; Sun et al. 2017). Consistent with these reports, our study showed that MeICT does not affect the mRNA expression of MMP-2. Although, as mentioned above, MeICT may interact with MMP-2 and decrease the secretion of MMP-2 that was not investigated here.
Although, ANXA2 protein was determined as a receptor for CTX, the roll of CTX on ANXA2 mRNA expression has not been investigated yet (Kesavan et al. 2009). In this study, we revealed the significant inhibitory effect of IMeCT on ANXA2 mRNA expression. ANXA2 is located in the pseudopodia of invasive glioma cells and binds to actin filaments of the cell membrane; therefore, its downregulation reduces cell migration (Zhai et al. 2011). Transcription of ANXA2 is regulated by external growth factors and kinase signaling pathways in cancer cells (Wang and Lin 2014). How MeICT can mediate these molecular pathways requires further study.

Due to the direct association between overexpression of FOXM1 and glioma progression, the expression of FOXM1 was also investigated in this study (Cheng et al. 2013). Depletion of FOXM1 reduces glioma progression and it has been recognized as a therapeutic target for this cancer (Halasi and Gartel 2013; Zhanget al. 2017). Here we reported down-regulation of FOXM1 mRNA level following MeICT treatment of glioma cells. Different mechanisms are involved in the regulation of FOXM1 that may contribute here (Liao et al. 2018). A previous report indicated that MMP-2 alters FOXM1 mRNA expression (Chetty et al. 2009). Therefore, MeICT may downregulate the expression of FOXM1 via interaction with MMP-2 and also decreasing MMP-2 secretion.

Both ANXA2 and FOXM1 mediate up-regulation of MMP-2 but by two distinct mechanisms (Dai et al. 2007; Sharma 2019). FOXM1 as a transcription factor binds to the MMP-2 promoter and increases its expression (Dai et al. 2007). Although FOXM1 was downregulated here, decrease in the MMP-2 mRNA level was not detected. It could be because of other regulation mechanisms that mediate MMP-2 transcription, for example different transcription factors bind to MMP-2 promoter including Sp1, YB-1, p53, and Stat3 (Pullen et al. 2018). On the other hand, ANXA2 regulates MMP-2 secretion via conversion of pro-MMP-2 to MMP-2. Studies have shown that downregulation of ANXA2 inhibits MMP-2 production and consequently inhibits invasion and angiogenesis of cancer cells (Zhao et al. 2010). Both CTX and BMKCT decrease the protein level of MMP-2 that does not correlate with MMP-2 gene expression and probably is related to decrease in MMP-2 secretion (El-Ghlban et al. 2014; Sun et al., 2017). We suggest that the decrease in MMP-2 production can be caused by ANXA2 downregulation. Therefore, CTX-like peptides probably affect MMP-2 through two distinctive mechanisms; binding directly to MMP-2 and inhibiting its activation in addition to, suppressing indirectly MMP-2 production by down-regulation of ANXA2.

Based on different studies about function of CTX-like peptides, we concluded that these peptides contain MeICT might display anti-glioma activity through different mechanisms, including interaction with chloride channel, MMP-2 and ANXA2, down-regulation of MMP-2 secretion and inhibition of ANXA2 and FOXM1 gene expression. The present study revealed that recombinant MeICT-His inhibits U87-MG glioma cell proliferation and migration. In vivo safety of MeICT and its potency to down-regulate expression of cancer markers such as ANXA2 and FOXM1 can make it a promising factor for glioma targeting.

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Author contributions MSG: investigation, methodology, visualization roles, writing original draft. HA: supervision, conceptualization, project administration, validation, writing, review and editing. AMA: data analysis, writing, review and editing.

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Declarations

Conflict of interest All authors declare they have no conflict of interest.

Ethical approval The permission for experimental animal procedures was obtained from Animal Ethics Committee of Shahrekord University.

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