# Growth Inhibition of MDA-MB-231 Cell Line by Peptides Designed based on uPA

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**Abstract-** Interaction between urokinase-type plasminogen activator (uPA) and its receptor (uPAR) plays an important role in the progression of numerous cancer types including breast cancer by promoting tumor initiating, proliferation, invasion and metastasis. Hence, disruption of this interaction inhibits their downstream cascades and subsequently tumor growth. For this, we created two series of 8 and 10 amino acids linear peptides, derived from uPA binding region to target uPAR and studied the inhibition of proliferation in MDA-MB-231 cell line. Results revealed that all of the 10-mer peptides inhibited breast cancer cell proliferation significantly with maximum 40% inhibition of 103 peptides. Meanwhile, none of the 8-mer peptides showed significant toxicity. Current results indicate that the linear 10-mer peptides which mimic a small part of a sequence of a binding domain of uPA to uPAR could be exploited to design a novel class of anti-cancer agents.



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**Keywords:** uPA; uPAR; Peptide; Growth inhibition; Cancer



## Introduction

Cancer is one of the leading causes of human death worldwide and due to its bio-molecular heterogeneity, it is crucial to developing novel therapies intended to target aberrant cell proliferation and treatment resistance. Among the array of factors executing indisputable roles in cancer development, uPA (Urokinase-type plasminogen activator) system has significance established function in formation and progression of malignancy via tumor initiation, proliferation, invasion, and metastasis (1-5). The uPA system consists of several components including serine proteases (uPA), serine inhibitors (PAI-1 and PAI-2) and uPA receptor (uPAR) (1,6). uPAR is a glycosylated cell surface protein of 50-60 KD covalently linked to the membrane via glycosyl phosphatidyl inositol (GPI) anchor (7-9). Many of uPAR activities are commenced through binding to its specific ligand; uPA (9,10). uPA is a serine protease that is synthesized as an inactive proenzyme (1,6,9,10). uPA is subdivided into three main domains; growth factor-like domain (GFD, aa1-49), kringle domain (aa50-135) and carboxyl-terminal domain (11). Binding of pro-uPA to uPAR produces active uPA which degrades extracellular matrix (ECM) leading to tumor migration not only by direct proteolysis but also by activation other proteases like matrix metalloproteinases (MMPs) (12,13). In addition to induction of uPA catalytic activity, uPAR initiates proliferative signals within cells through uPA binding as well (14,15).

Aside from MMPs and uPA activation, uPAR enhances oncogenic properties of cancer cells by several other mechanisms (16-22). The MEK/ERK signaling pathway is one of the downstream cascades of uPAR which governs proliferation, differentiation and cell survival (16,23-26). ERK activation is often deregulated in many cancer types as its constitutive activation enhances cell migration, metastasis and proliferation

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(27). ERK hyper-activation could also disturb the counter balance of Bcl-2 family members in favor of cell survival (28).

Collectively, all above studies highlight the significance of activation of the uPAR system in cell proliferation and subsequently introduce uPAR as a potential target for cancer treatment. In this regard, we aimed to examine the effects of some newly designed peptides as an inhibitor of uPAR on cell proliferation. We designed a series of 8 and 10 amino acids synthetic linear peptides using conserved residues of uPA GFD domain and examined their capability of modulating cell proliferation and cytotoxicity.

## Materials and Methods

### Peptide sequences and treatment

Our putative antagonists fell into two categories of 8 and 10 amino acid peptides. The sequences of 8 amino acids (8-mer) peptides were: SQKYFSYI (peptide 81), STKYFSWI (peptide 82), SQKYFSRI (peptide 83) and SFKYFSDI (peptide 84) and the sequences of 10 amino acids (10-mer) were: SNKYFTRIRW (peptide 101), SQKYFTQIYR (peptide 102), SYKYFTQIHY (peptide 103) and SNKYFSNIRR (peptide 104). All of these peptides were synthesized by GenScript (USA). According to the manufacturer's protocol, lyophilized peptides were dissolved in sterile water at pH=7 except for SFKYFSDI, which was dissolved in water at pH=4. Peptides were stored as a 10-mM stock solution and kept at -20°C.

### Cell culture

MDA-MB-231, human breast cancer cell line was obtained from German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Cells were cultured to 70% confluency in high glucose Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum(FBS), 100 units/ml penicillin and 100 µg/ml streptomycin (all from PAA, Austria) in a humidified atmosphere containing 5% CO2 at 37°C. Cells were subcultured at a 1: 4 split ratio every 2 days using 1X Trypsin-EDTA (PAA, Austria).

### Cell proliferation assay

The effects of peptides on the proliferation of MDA-MB-231 cell line were determined by MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Sigma-Aldrich, Dorset, UK). Briefly, the cells were seeded at the final concentration of 10000cells/well in a 96-well tissue culture plate. The day after, the medium was exchanged with medium containing 50, 100, 250 and 500µM of peptides (4 replicates/ experiment). After 24 hours, the medium was removed, and 20µl from 5mg/ml MTT dissolved in PBS added per well and plates were incubated for 4 hours. Formazan crystals were dissolved by adding 60µl DMSO (dimethyl sulfoxide) to each well. Absorbance was measured at 570nm by Biotek microplate reader (USA) with a 690nm reference for background correction.

### Statistical analysis

Data were expressed as mean±SE of three independent experiments and statistical evaluations were performed by One-way ANOVA and Bonferroni's Multiple Comparison post-test. *P*-values less than 0.05 were considered to be significant.

## Results

### The 10 amino acid peptides inhibited proliferation of MDA-MB-231 cell line

To determine the effects of the peptides on the cell growth, MDA-MB-231 human breast cancer cell line was used in our experiments. This cell line harbors high levels of uPA system components including uPA and uPAR and is thus a suitable model to examine the efficiency of our peptides. Cells were treated with above-mentioned concentrations of 10 amino peptides and subjected to MTT assay after 24 hours. Results revealed that all of the 10-mer peptides had significant toxicity at the employed doses; however, we did not observe a concentration-dependent inhibition in the doses we used (Figure 1). Meanwhile, among all of tested peptides, peptide 103 was the most efficacious one in cytotoxicity induction.

### The 8 amino acid peptides did not show significant toxicity in MDA-MB-231 cell line

As shown in Figure 2, Based on MTT cytotoxicity/ proliferation assay results among 8 amino acid peptides, none of them has mediated any significant growth inhibition or cytotoxicity compared to control. Moreover, there is not any variation even by dose enhancement.

## Discussion

The uPA receptor mediates various cancer development processes including inflammation, metastasis, invasion, angiogenesis and cell proliferation via binding to its specific ligand, uPA (9,10). Accordingly, designing antagonists against uPAR capable of binding and intervening downstream

signaling might be beneficial for cancer therapy.

Here, we built present study on creating antagonist peptides which mimic the critical part of uPA; GFD, for binding to its receptor. This domain encompasses two different regions responsible for attachment. Region 1 contains conserved amino acid residues in uPA such as Lys23, Tyr24, Phe25 and Ile28 whereas amino acids in region 2 account for determining species specificity (29). We retained the conserved amino acids in designing linear synthetic peptides in which the sequence of 8-mer peptides is similar to region 1, but the 10-mer ones contain both regions.

Current data showed that 8 amino acid peptides are not cytotoxic for MDA-MB-231 whereas, adding 2 more amino acids resulted in opposite effects. Thus, 10mer peptides demonstrated cytotoxicity in these cells. This could be in part due to the importance of region 2 of uPA in its attachment. Besides, remodeling of uPAR confirmation followed by binding to these peptides could be another justification for these diverse results. uPAR is comprised of three domains joined together to create the large hydrophobic cavity (30). Binding of the main ligand of uPAR; uPA, to this central cavity increases the affinity between uPAR and other cell surface proteins like vitronectin (31-33). Although it may inhibit uPA binding, 8-mer peptides can induce conformational changes in uPAR leading to its interplay with other ligands such as vitronectin, which are in turn responsible for cancer progression and proliferation.

Accordingly, further in vitro and in vivo research could provide evidence to introduce the suitable novel drug in cancer therapy based on 10-mer uPA peptides.

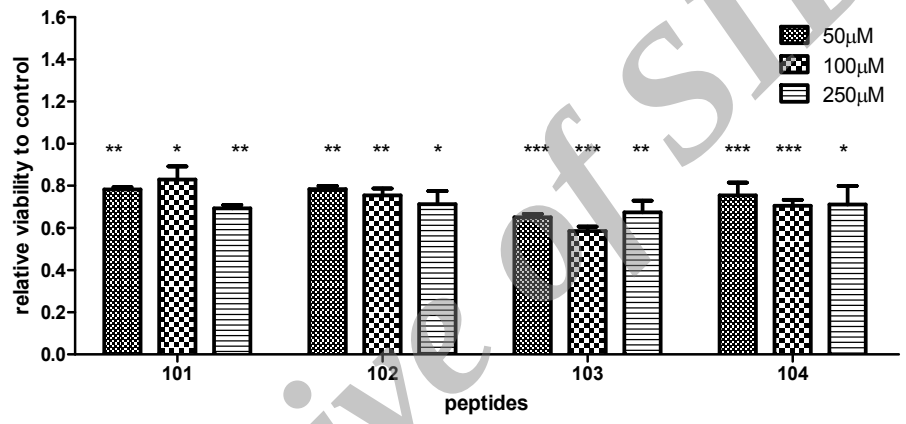


**Figure 1**.The cytotoxicity evaluation of 10 mer peptides on MDA-MB-231. The cells were treated with 10 amino acid peptides for 24h. After which cell proliferation was measured by MTT assay. SNKYFTRIRW (101), SQKYFTQIYR (102), SYKYFTQIHY (103), and SNKYFSNIRR (104). Data are calculated as relative to control. Results represent the means ± SE of three different experiments (n=3, **P*<0.05; ***P*<0.01; ****P*<0.001 compared to control)

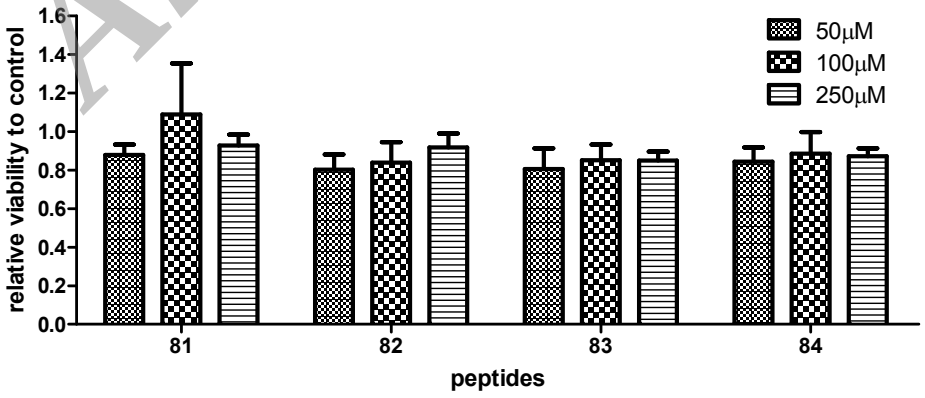


**Figure 2.**The cytotoxicity evaluation of 8mer peptides on MDA-MB-231. The cells were treated with 8 amino acid peptides for 24h. After which cell proliferation was measured by MTT assay. SQKYFSYI (81), STKYFSWI (82), SQKYFSRI (83) and SFKYFSDI (84). Data are calculated as relative to control. Results represent the means ± SE of three different experiments (n=3)

## References


1. Duffy MJ. The urokinase plasminogen activator system: role in malignancy. Curr Pharm Des 2004;10(1):39-49.
2. Aref S, El-Sherbiny M, Mabed M, et al. Urokinase plasminogen activator receptor and soluble matrix metalloproteinase-9 in acute myeloid leukemia patients: a possible relation to disease invasion. Hematology 2003;8(6):385-91.
3. Foekens JA, Peters HA, Look MP, et al. The urokinase system of plasminogen activation and prognosis in 2780 breast cancer patients. Cancer Res 2000;60(3):636-43.
4. Meijer-van Gelder ME, Look MP, Peters HA, et al. Urokinase-type plasminogen activator system in breast cancer: association with tamoxifen therapy in recurrent disease. Cancer Res 2004;64(13):4563-8.
5. Rigolin GM, Tieghi A, Ciccone M, et al. Soluble urokinase-type plasminogen activator receptor (suPAR) as an independent factor predicting worse prognosis and extra-bone marrow involvement in multiple myeloma patients. Br J Haematol 2003;120(6):953-9.
6. Andreasen PA, Kjoller L, Christensen L, et al. The urokinase-type plasminogen activator system in cancer metastasis: a review. Int J Cancer 1997;72(1):1-22.
7. Mazar AP. The urokinase plasminogen activator receptor (uPAR) as a target for the diagnosis and therapy of cancer. Anticancer Drugs 2001;12(5):387-400.
8. Huai Q, Mazar AP, Kuo A, et al. Structure of human urokinase plasminogen activator in complex with its receptor. Science 2006;311(5761):656-9.
9. Alfano D, Franco P, Vocca I, et al. The urokinase plasminogen activator and its receptor: role in cell growth and apoptosis. Thromb Haemost 2005;93(2):205-11.
10. Ploug M. Structure-function relationships in the interaction between the urokinase-type plasminogen activator and its receptor. Curr Pharm Des 2003;9(19):1499-528.
11. Appella E, Robinson EA, Ullrich SJ, et al. The receptor-binding sequence of urokinase. A biological function for the growth-factor module of proteases. J Biol Chem 1987;262(10):4437-40.
12. Mazzieri R, Blasi F. The urokinase receptor and the regulation of cell proliferation. Thromb Haemost 2005;93(4):641-6.
13. Wang Y. The role and regulation of urokinase-type plasminogen activator receptor gene expression in cancer invasion and metastasis. Med Res Rev 2001;21(2):146-70.
14. Gyetko MR, Todd RF 3rd, Wilkinson CC, et al. The urokinase receptor is required for human monocyte chemotaxis in vitro. J Clin Invest 1994;93(4):1380-7.
15. Busso N, Nicodeme E, Chesne C, et al. Urokinase and type I plasminogen activator inhibitor production by normal human hepatocytes: modulation by inflammatory agents. Hepatology 1994;20(1 Pt 1):186-90.
16. Aguirre Ghiso JA, Kovalski K, Ossowski L. Tumor dormancy induced by downregulation of urokinase receptor in human carcinoma involves integrin and MAPK signaling. J Cell Biol 1999;147(1):89-104.
17. Aguirre Ghiso JA. Inhibition of FAK signaling activated by urokinase receptor induces dormancy in human carcinoma cells in vivo. Oncogene 2002;21(16):2513-24.
18. Vial E, Sahai E, Marshall CJ. ERK-MAPK signaling coordinately regulates activity of Rac1 and RhoA for tumor cell motility. Cancer Cell 2003;4(1):67-79.
19. Liu D, Aguirre Ghiso J, Estrada Y, et al. EGFR is a transducer of the urokinase receptor initiated signal that is required for in vivo growth of a human carcinoma. Cancer Cell 2002;1(5):445-57.
20. Kjoller L, Hall A. Rac mediates cytoskeletal rearrangements and increased cell motility induced by urokinase-type plasminogen activator receptor binding to vitronectin. J Cell Biol 2001;15296):1145-57.
21. Koshelnick Y, Ehart M, Hufnagl P, et al. Urokinase receptor is associated with the components of the JAK1/STAT1 signaling pathway and leads to activation of this pathway upon receptor clustering in the human kidney epithelial tumor cell line TCL-598, J Biol Chem 1997;272(45):28563-7.
22. Sidenius N, Blasi F. The urokinase plasminogen activator system in cancer: recent advances and implication for prognosis and therapy, Cancer Metastasis Rev 2003;22(2-3):205-22.
23. Kanse SM, Benzakour O, Kanthou C, et al. Induction of vascular SMC proliferation by urokinase indicates a novel mechanism of action in vasoproliferative disorders. Arterioscler Thromb Vasc Biol 1997;17(11):2848-54.
24. Tang H, Kerins DM, Hao Q, et al. The urokinase-type plasminogen activator receptor mediates tyrosine phosphorylation of focal adhesion proteins and activation of mitogen-activated protein kinase in cultured endothelial cells. J Biol Chem 1998;273(29):18268-72.
25. Konakova M, Hucho F, Schleuning WD. Downstream targets of urokinase-type plasminogen-activator-mediated signal transduction. Eur J Biochem 1998;253(2):421-9.
26. Nguyen DH, Catling AD, Webb DJ, et al. Myosin light chain kinase functions downstream of Ras/ERK to promote migration of urokinase-type plasminogen activator-stimulated cells in an integrin-selective manner. J Cell Biol 1999;146(1):149-64.
27. Cho SY, Klemke RL. Extracellular-regulated kinase activation and CAS/Crk coupling regulate cell migration

and suppress apoptosis during invasion of the extracellular matrix. J Cell Biol 2000;149(1):223-36.
28. Alfano D, Iaccarino I, Stoppelli MP. Urokinase signaling through its receptor protects against anoikis by increasing BCL-xL expression levels. J Biol Chem 2006;281(26):17758-67.
29. Lin L, Gardsvoll H, Huai Q, et al. Structure-based engineering of species selectivity in the interaction between urokinase and its receptor: implication for preclinical cancer therapy. J Biol Chem 2010;285(14):10982-92.
30. Barinka C, Parry G, Callahan J, et al. Structural basis of interaction between urokinase-type plasminogen activator and its receptor. J Mol Biol 2006;363(2):482-95.
31. Huai Q, Zhou A, Lin L, et al. Crystal structures of two human vitronectin, urokinase and urokinase receptor complexes. Nat Struct Mol Biol 2008;15(4):422-3.
32. Gardsvoll H, Ploug M. Mapping of the vitronectin-binding site on the urokinase receptor: involvement of a coherent receptor interface consisting of residues from both domain I and the flanking interdomain linker region. J Biol Chem 2007;282(18):13561-72.
33. Sidenius N, Andolfo A, Fesce R, et al. Urokinase regulates vitronectin binding by controlling urokinase receptor oligomerization. J Biol Chem 2002;277(31):27982-90.