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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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 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.
Cytotoxicity of uPAR peptide antagonist

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 8-mer 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).
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References

27. Cho SY, Klemke RL. Extracellular-regulated kinase activation and CAS/Crk coupling regulate cell migration.