Structure-activity relationship of HER2 receptor targeting peptide and its derivatives in targeted tumor therapy

Afrodité Emese Adorján[1,2], Ildikó Szabó[2], Szilvia Bősze[2], Gábor Mező[1,2]

[1]*Eötvös Loránd University (ELTE), Faculty of Science, Institute of Chemistry*

[2]*MTA-ELTE, Research Group of Peptide Chemistry, Budapest, Hungary*

gmezo@elte.hu

INTRODUCTION

In normal cells, epidermal growth factor receptor 2 (HER2/ErbB2) plays a vital role in various cellular processes and the expression level of HER2 remains stable. When overexpression of HER2 occurs it can disrupt the dynamic balance of many cellular processes and lead to uncontrollable tumor growth [1], because: (a) overexpression makes excessive HER2 receptors available to form extra heterocomplexes, (b) HER2 may strengthen the affinity of ligand-binding for other receptors, (c) HER2 might weaken the specificity of its heterodimerization partners, (d) HER2 engaged dimerization can activate proliferation and survival, (e) HER2-containing heterodimers may escape from the internalization or degradation of HER2 dimers.

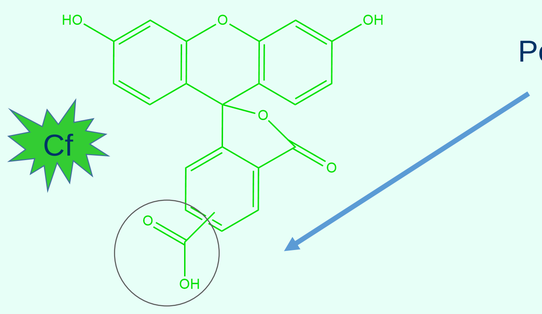
Breast cancer is one of the most leading causes of death worldwide. In 20% of all cases HER2 is found on breast cancer tissues. The overexpression of HER2 leads to aggressive course of disease with poor prognosis. Therefore, new, efficient therapeutic approaches are needed. For this purpose, targeted tumor therapy is a promising tool to increase selectivity of antitumor drug attached to a targeting molecule, which binds to tumor specific antigens/receptors. Since HER2 might be a good target to prevent tumor growth, current researches are directed to discover HER2 targeting moieties for drug delivery. Small molecule – drug conjugates (SMDCs) based on peptides as targeting moieties might have advantages over antibody – drug conjugates. Appropriate HER2 specific homing peptides have been selected by phage displays (e.g. KCCYSL [2]) or molecular dynamic (MD) simulations (e.g. GYYNPT [3]).

AIMS

1. Synthesis of 5(6)-carboxyfluorescein (Cf) derivatives of KCCYSL and GYYNPT peptides;
2. Sequence optimization of the Cf-labeled peptides;
3. Combination of the two different peptides in one targeting moiety;
4. Study of the cellular uptake of the Cf-labeled peptides by different type of cancer cells;
5. Determination of structure-activity relationship.

SYNTHESIS

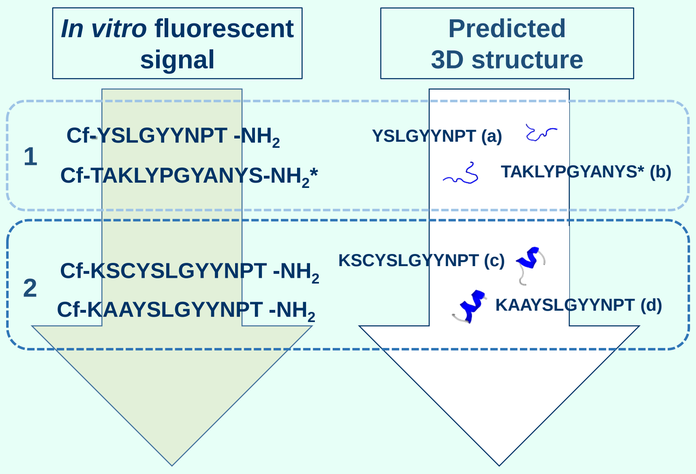
Peptides were prepared by SPPS on Rink Amide MBHA resin using Fmoc/^{t}Bu protocol. At the last step, Cf as fluorescent labelling was atteched to the *N*-terminus of the peptides.



Peptides: KAAYSL-NH_2
KSCYSL-NH_2
GYYNPT-NH_2
YSLGYYNPT-NH_2
KAAYSLGYYNPT-NH_2
KSCYSLGYYNPT-NH_2

Caracterization: analytical RP-HPLC, ESI-MS

PREDICTION AND DISCUSSION



Group 1: random coil (a) and quite low helical structure (b)

NO FLUORESCENT SIGNAL

Group 2: helix formation (c,d)

HIGH FLUORESCENT SIGNAL AND BINDIG ABILITY ON CELL SURFACE

- Helicity was predicted by PEP-FOLD3.5 method
- YSLGYYNPT (random coil) does not form helical structure at all, while the probability of helix formation is quite low for peptide with scrambled sequence TAKLYPGYANYS
- helix formation is highly feasible for peptides KAAYSLGYYNPT and KSCYSLGYYNPT and their binding ability is high
- this observation was in accordance with the structural features of an engineered HER2 binding affibody containing three helical parts [4]

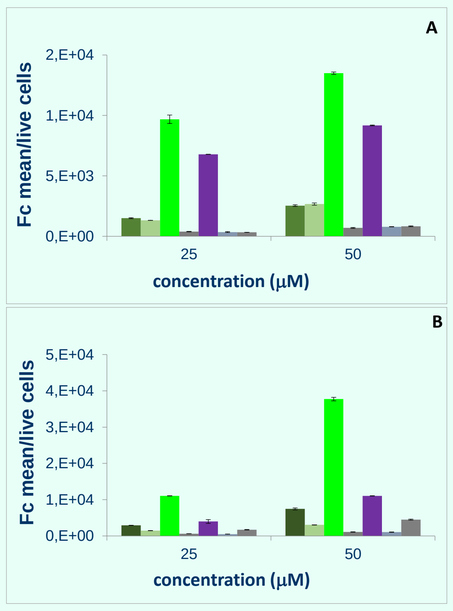
CONCLUSION

1. Selection of peptide sequences by phage display and/or molecular dynamic simulations is a good tool for the development of conjugates for targeted tumor therapy.
2. In contrast to the Cf-labelled predicted hexapeptides, the designed combined peptides Cf-KAAYSLGYYNPT and Cf-KSCYSLGYYNPT showed high tumor localization. Nevertheless, our data suggest that the combined peptide suitable for receptor binding with high affinity, but the internalization rate of the peptides is low.
3. Therefore, these promising designed homing peptides may be applied for tumor diagnostic (e.g. PET) or selective delivery of radiotracers with therapeutic activity.
4. In addition, the application of extracellular enzyme (elastase, MMPs) cleavable spacers between the homing peptide and an antitumor agent might be a good choice for the development of conjugates as drug delivery systems.

RESULTS AND DISCUSSION

➢ *In vitro* cellular uptake

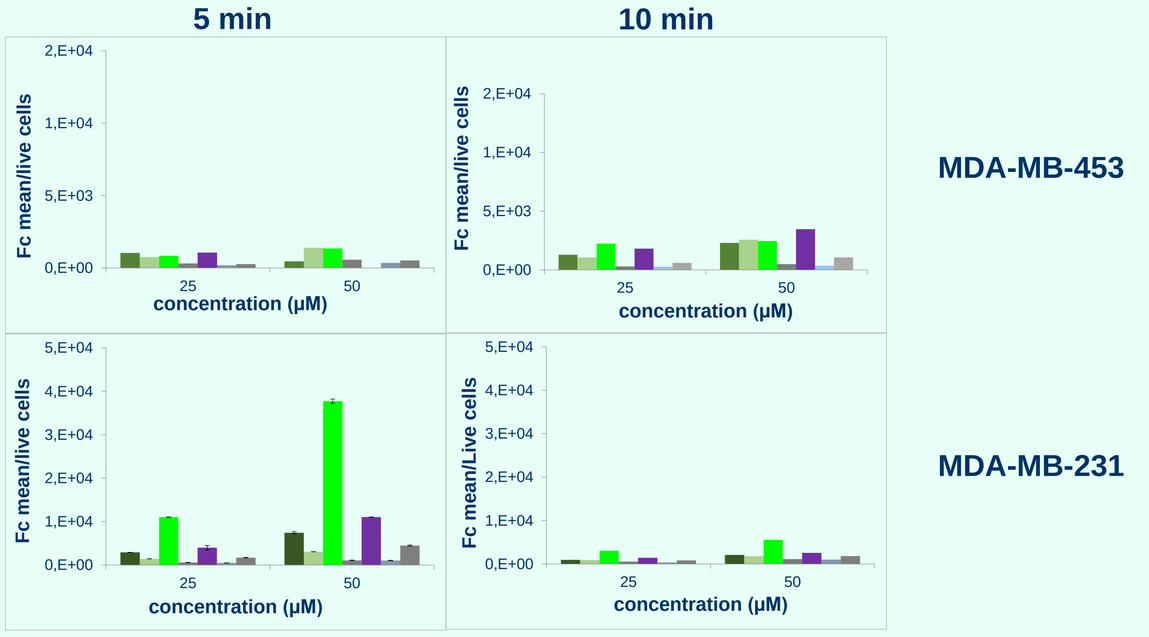
Cellular uptake was determined on MDA-MB-453 human breast metastatic carcinoma (A) and MDA-MB-231 (B) human breast adenocarcinoma cells using flow cytometry (BD LSR II, λ= 488 nm, 3h treatment).



Cf-KAAYSLGYYNPT-NH_2
Cf-KSCYSLGYYNPT-NH_2
Cf-YSLGYYNPT-NH_2
Cf-GYYNPT-NH_2
Cf-KSCYSLGYYNPT-NH_2
Cf-YSLGYYNPT-NH_2
Cf-TAKLYPGYANYS-NH_2 – scrabled control peptide

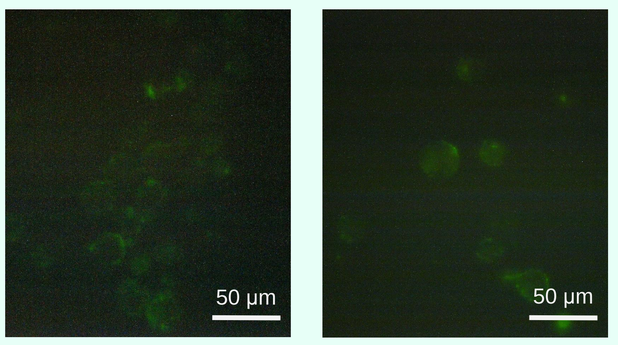
- according to flow cytometry analysis the fluorescent signal intensity of Cf-KAAYSLGYYNPT, Cf-KSCYSLGYYNPT was the highest
- fluorescent signal mainly was detected on the cell membrane/cell surface, and a small amount of signal concentrated on the cytoplasm.
- fluorescent signal significantly was decreased using longer trypsin treatment

- **Trypsinization**



➢ Fluorescent microscopy

To visualize MDA-MB-231 cell morphology, after the Cf-peptide treatment, microscopic images of cells were captured (Olympus CKX41 microscope, 50 μM final concentration, 3h treatment).



Cf-KAAYSLGYYNPT-NH_2 Cf-KSCYSLGYYNPT-NH_2

fluorescent signal mainly was concentrated on the cell membrane, and a small amount of signal was detected in the cytoplasm

REFERENCES AND ACKNOWLEDGEMENT

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