Targeted destruction of FSHR-positive cells by lytic peptide Phor21 conjugated with FSHβ subunit *in vitro*

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INTRODUCTION

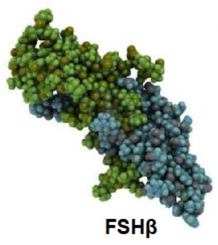
Follicle stimulating hormone receptor (FSHR) expression has been shown in gonadal tumors, as well as in endothelial tumor vessel cells of various cancers. FSHR, due to its transmembrane localization could be a good candidate for receptor-mediated targeted cancer therapy. In recent years, a number of membrane disrupting lytic peptides have been successfully used for receptor-based cancer therapy.

Aim of this study was to:

1. design and synthesize lytic peptides Phor21 conjugated to three different amino-acid (AA) sequence fragments of the human FSHβ-chain (AA33–53, AA81-95 and AA33-53+81-95);
2. establish a reproducible and functionally characterized *in vitro* model stably expressing hFSHR;
3. test the specificity and cytotoxicity of a Phor21-FSHβ conjugates to FSHR-positive cells *in vitro*;
4. validate the optimal conjugate structure for future *in vivo* studies.

METHODS

1 **Design, synthesis and purification of the Phor21-FSHβ conjugates**

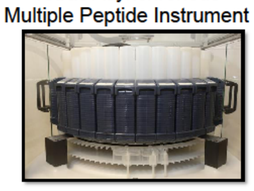


Bioinformatical design

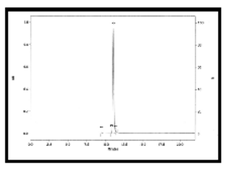
Phor21 KFAKFAKKFAKFAKKFAKFAK
FSHβ33-55 YTRDLVYKDPARPKIQKTCTF
FSHβ81-95 QCHCGKCDSDSTDCT

Phor21-FSHβ33-55 C-S or C-A or C-(Amc) and $CONH_2$
Phor21-FSHβ81-95 C-S or C-A or C-(Amc) and $CONH_2$
Phor21-FSHβ33-55+FSHβ81-95 C-S or C-A or C-(Amc) and $CONH_2$

Synthesis
Tetras Asynchronous Multiple Peptide Instrument

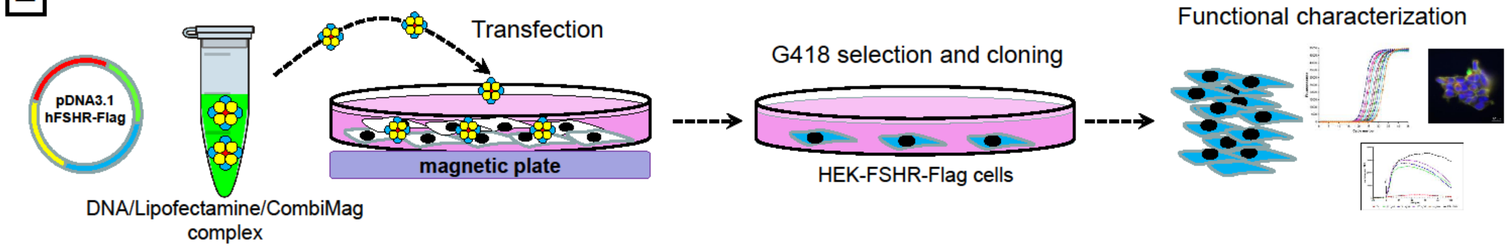


Purification
HPLC-MS-Analysis

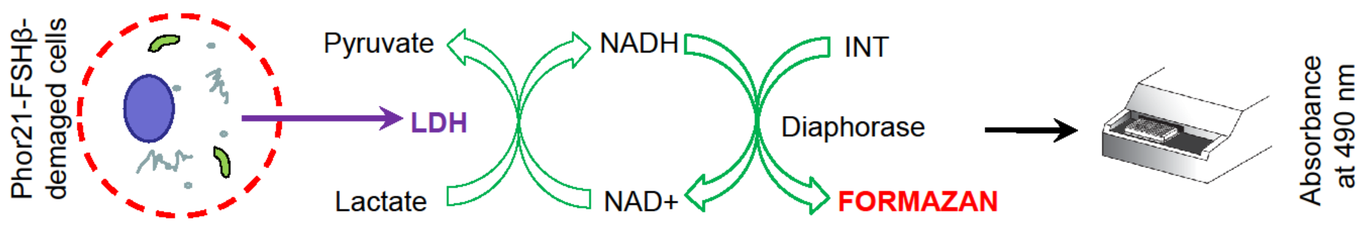


Peptides&Elephants

2 **HEK-FSHR-Flag cells – *in vitro* model**

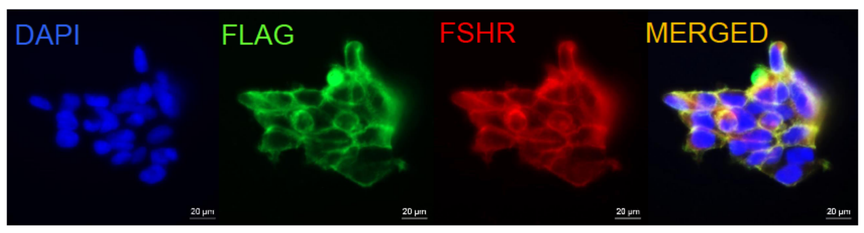


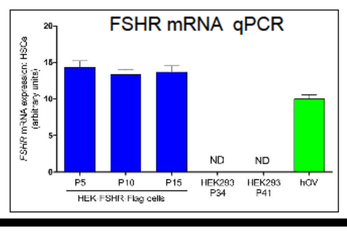
3 **Lactate dehydrogenase (LDH) release-based cytotoxicity assay**

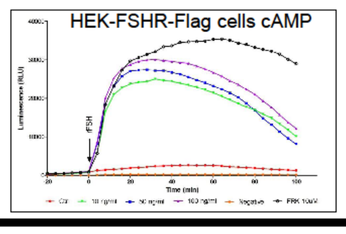


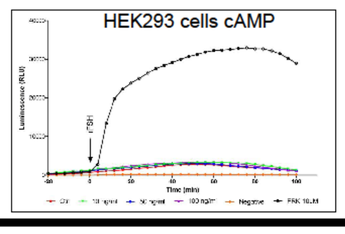
RESULTS

A **HEK-FSHR-Flag cells – *in vitro* model**

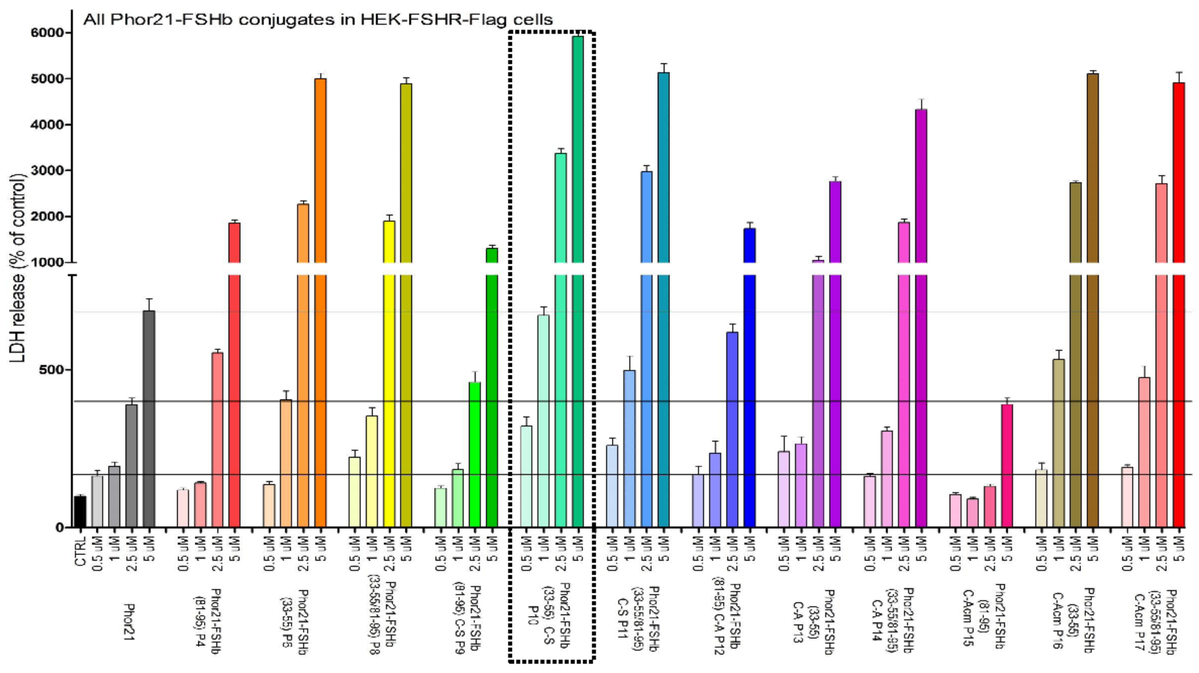








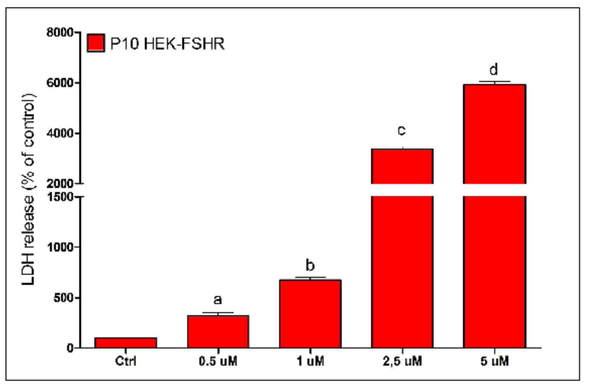
B **Primary screaning of the most cytotoxic conjugates**



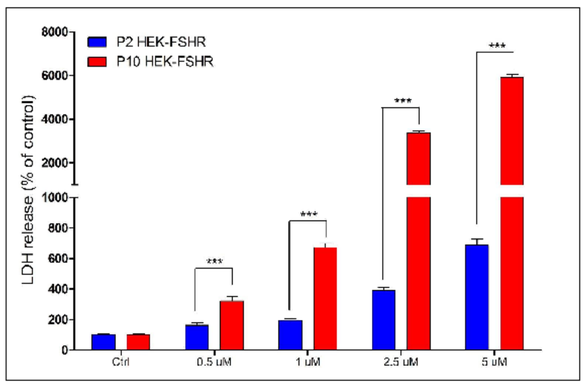
C **Detailed characterization of selected conjugate Phor21-FSHβ33-53Cys/Ser-$CONH_2$ (P10)**

KFAKFAKKFAKFAKKFAKFAKYTRDLVYKDPARPKIQKTSTF-$CONH_2$

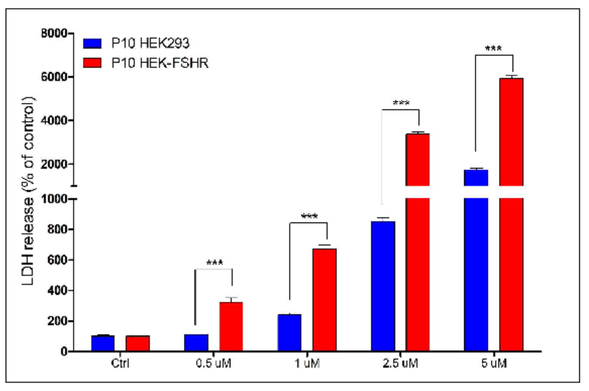
Phor21 **FSHβ**



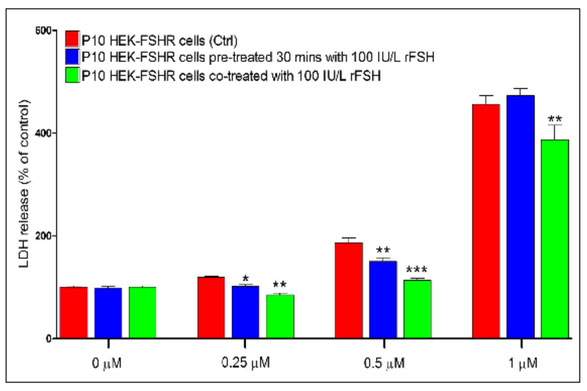
Dose-response for Phor21-FSHβ33-53Cys/Ser-$CONH_2$–induced cytotoxicity in HEK-FSHR-Flag cells.



Comparison of Phor21 *vs.* Phor21-SHβ33-53Cys/Ser-$CONH_2$ cytotoxicity in HEK-FSHR-Flag cells



Comparison of HEK293 *vs.* HEK-FSHR-Flag cells sensitivity to cytotoxic dose-response of Phor21-FSHβ33-53Cys/Ser-$CONH_2$.



Phor21-FSHβ33-53Cys/Ser-$CONH_2$–induced cytotoxicity in HEK-FSHR-Flag cells pre-, or co-treated with 100 IU/L rFSH.

CONCLUSION

Phor21-FSHβ33-53Cys/Ser-$CONH_2$ conjugate provides a novel selective therapeutic lead into targeted destruction of FSHR expressing cells *in vitro* and as well as for further *in vivo* studies.

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