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SEQUENCE OPTIMIZATION OF HOMING PEPTIDE (VHLGYAT) SELECTED FOR HT-29 COLON CANCER BY PHAGE DISPLAY

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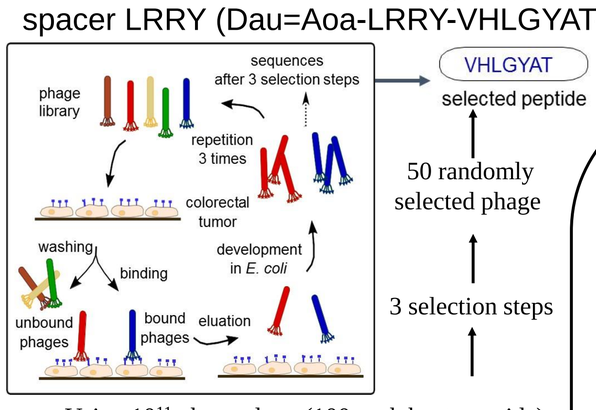
INTRODUCTION

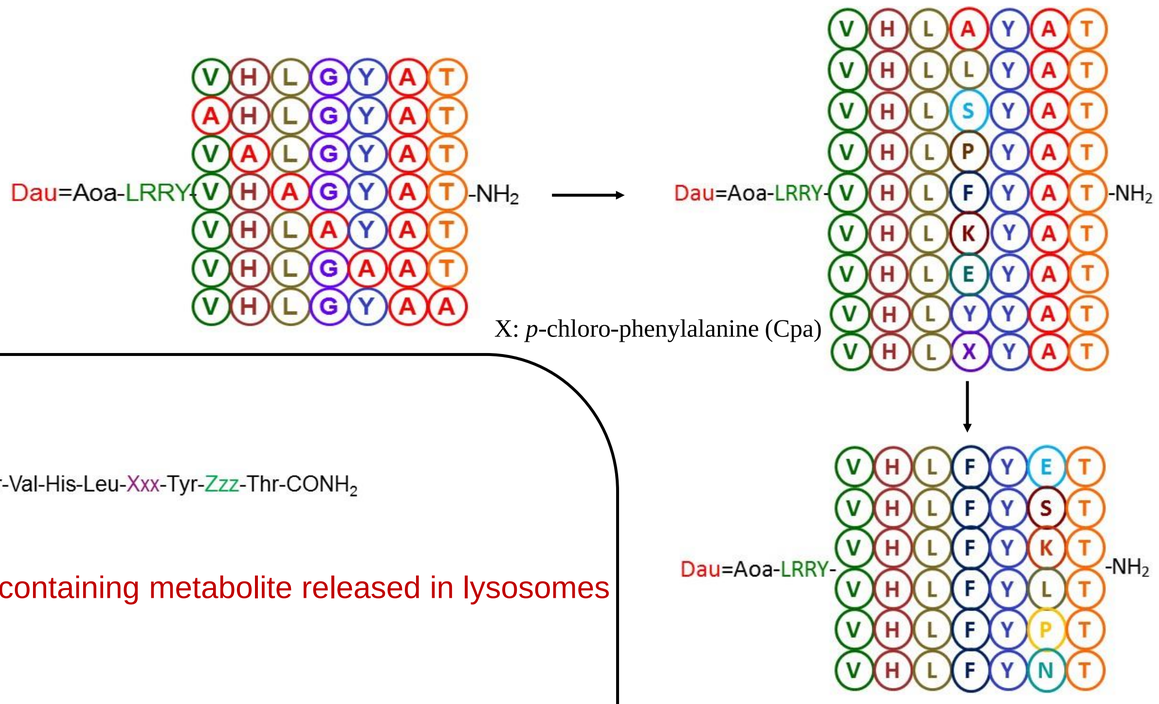
Colorectal cancer is the third most common type of cancer worldwide [1]. Therefore, the development of efficient therapeutic strategies is of utmost importance. Peptide-based targeted tumor therapy, which has been investigated in the last decades, might be an effective therapeutic approach to cure colon cancer as well. Its principle relies on the structural and/or functional differences between cancer cells and healthy ones. One of the possible approaches is based on the attachment of an anticancer drug to a peptide based targeting moiety, which recognizes tumor specific receptors or cell surface structures that are highly expressed on tumor cells. A peptide sequence (VHLGYAT) selected for HT-29 colon cancer cell line by phage display [2] was chosen to our study. Daunomycin was attached via oxime linkage to the homing peptide trough an enzyme labile spacer LRRY (Dau=Aoa-LRRY-VHLGYAT-NH_2).

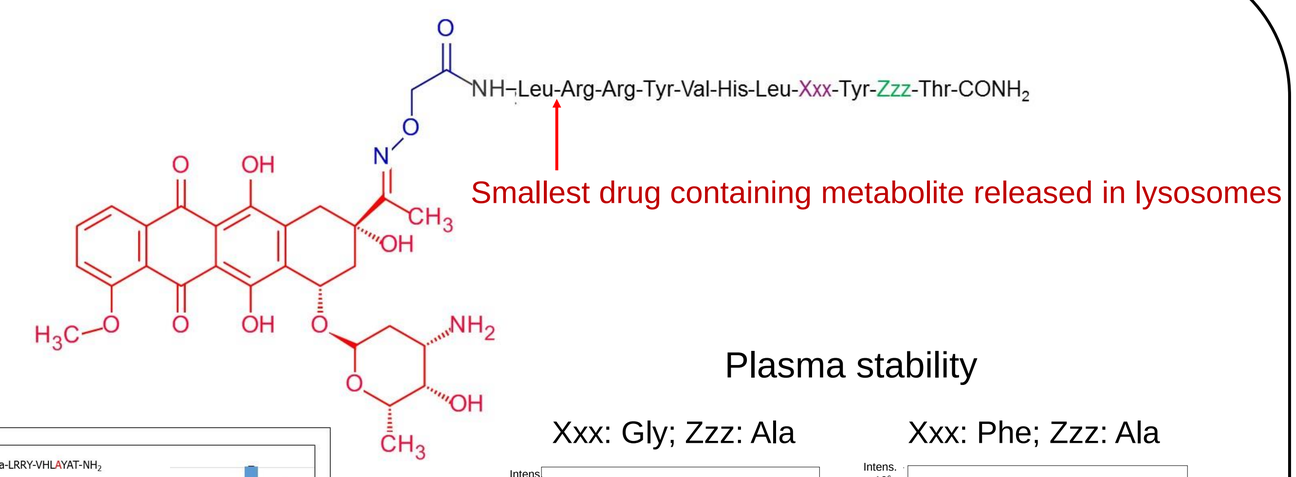


Using 10^{11} phage clone (100 each heptapeptide)

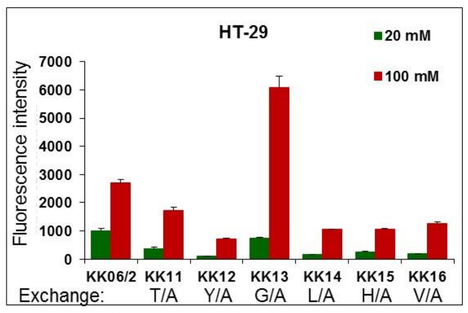
AIMS

1. Optimization the sequence of homing peptide by Ala-scan followed by positional scanning;
2. Study of *in vitro* cytostatic effects, cellular uptake and stability
3. *In vivo* experiments on orthotopically developed HT-29 tumor bearing SCID mice

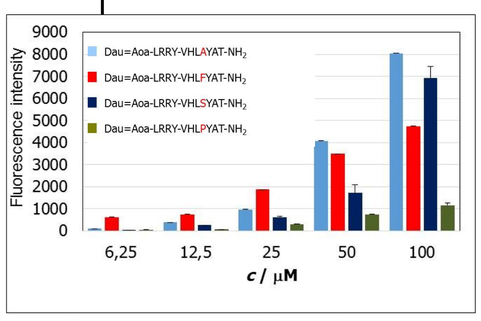




Cellular uptake studies



Influence of Ala-scan

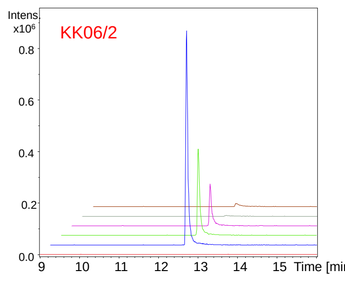


Influence of positional-scan

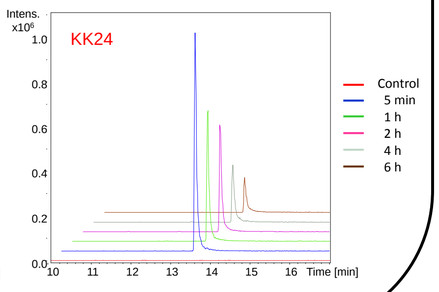
Cellular uptake was measured on HT-29 colon cancer cells after 3 h incubation.
Fluorescence intensity was detected on living cells.

Plasma stability

Xxx: Gly; Zzz: Ala



Xxx: Phe; Zzz: Ala



In vivo cytostatic effect

Treatment in 2% serum containing RPMI cell culture medium for 24 hrs followed by 48 hrs further incubation in fresh serum containing medium.

Selection of the most active compounds on HT-29 cells (IC_{50} in μM):

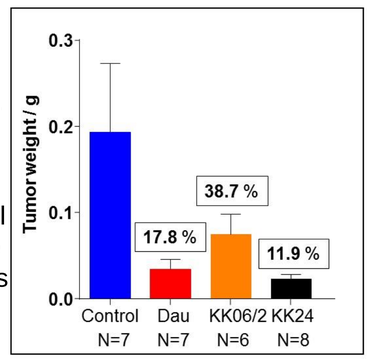
Dau=Aoa-LRRY-VHLGYAT-NH_2 (KK06/2)	50.5±5.5
Dau=Aoa-LRRY-VHL**A**YAT-NH_2	14.0±1.5
Dau=Aoa-LRRY-VHL**L**YAT-NH_2	7.5±3.5
Dau=Aoa-LRRY-VHL**F**YAT-NH_2 (KK24)	6.5±0.3
Dau=Aoa-LRRY-VHL*Cpa*YAT-NH_2	3.6±0.1
Dau=Aoa-LRRY-VHL**F**Y**L**T-NH_2	3.2±0.1

In vivo experiments

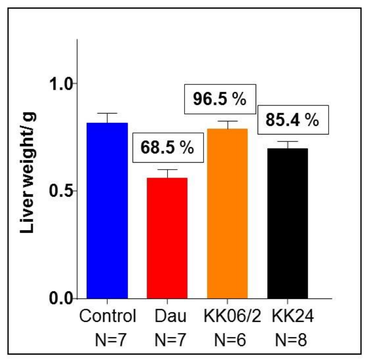
Treatments (*i.p.*):
First treatment was 10 days after the tumor transplantation.
Dau: 1mg/kg once a week;
Conjugates: 10 mg/kg Dau cont. 3 times on week 1 and twice on week 2.

1-1 mouse died from the control and Dau treated and two mice from the KK06/2 treated groups during the experiment.

Tumor growth inhibition



Liver toxicity



Type of tumors (cell lines)	Dau (IC_{50}, μM)	KK06/2 (IC_{50}, μM)	KK06/2 / Dau	KK24 (IC_{50}, μM)	KK24 / Dau	KK06/2 / KK24
Melanoma (mice B16)	0.0089 ± 0.0053	15.2 ± 3.0	1711.3	3.0 ± 0.5	335.4	5.07
Prostate (DU145)	0.0245 ± 0.0053	6.1 ± 2.2	249.7	3.5 ± 0.5	142.3	1.74
Lung (H6509)	0.0475 ± 0.0016	4.4 ± 1.2	92.6	2.9 ± 0.6	60.1	1.52
Lung (H1975)	0.0133 ± 0.0047	19.3 ± 0.1	1458.7	3.7 ± 0.8	279.9	5.22
Melanoma (A2058)	0.0332 ± 0.0004	10.5 ± 5.8	316.8	3.5 ± 1.3	104.5	3.00
Head & neck (PE/CA PJ41)	0.0258 ± 0.0054	9.4 ± 3.5	363.9	4.3 ± 0.1	165.7	2.19
Head & neck i (PE/CA PJ15)	0.0264 ± 0.0050	20.2 ± 4.6	759.3	7.4 ± 3.4	277.3	2.73
Liver(HepG2)	0.0213 ± 0.0009	22.4± 4.4	1052.6	4.7 ± 0.5	220.2	4.77
Melanoma (M24)	0.0936 ± 0.0258	15.4 ± 3.7	164.3	5.8 ± 0.9	61.7	2.66
Breast (MDA-MB-231)	0.0529 ± 0.0103	6.3 ± 2.5	118.8	4.6 ± 0.8	86.6	1.37
Melanoma (WM983b)	0.0442 ± 0.0192	7.1 ± 2.5	159.8	5.1 ± 0.4	114.6	1.39
Glioma (U87MG)	0.0279 ± 0.0035	14.2 ± 3.5	510.3	6.6 ± 0.2	236.9	2.15
Lung (A549)	0.0681 ± 0.0227	25.9 ± 2.3	380.1	5.9 ± 1.5	86.1	4.39
Colorectal (HT116)	0.1271 ± 0.0219	33.6 ± 4.4	264.1	7.2 ± 0.3	57.0	4.67
Prostate (PC-3))	0.0260 ± 0.0071	20.5 ± 0.3	787.5	5.9 ± 1.6	226.7	3.48
Colorectal (egér C26)	0.1260 ± 0.0468	15.8 ± 2.5	125.7	8.3 ± 1.0	66.0	1.90
Colorectall (HT-29)	0.2029 ± 0.0010	30.1 ± 0.3	148.3	11.6 ± 0.1	57.1	2.60
Colorectal (WIDR)	0.2401 ± 0.0363	34.1 ± 3.2	141.8	15.1 ± 2.9	62.8	2.26
Ovarian (OVCAR-3)	0.4729 ± 0.0636	13.8 ± 0.5	29.3	11.3 ± 2.6	24.0	1.22
Breast (MCF-7)	0.2860 ± 0.0247	22.2 ± 9.2	77.6	11.1 ± 3.8	38.8	2.00
Breast (mice 4T1)	0.0408 ± 0.0068	34.2 ± 0.4	837.4	11.6 ± 3.6	284.6	2.95
Colorectal (HT-25)	0.1564 ± 0.0721	33.2 ± 3.5	212.4	15.4 ± 2.8	98.5	2.16
Pancreas (PANC-1)	0.4667 ± 0.0366	31.7 ± 4.5	68.0	26.9 ± 8.1	57.7	1.18
Fibroblast (MRC-5)	0.2547 ± 0.0006	39.3 ± 24.6	154.2	52.1 ± 1.7	204.6	0.75

Observations:

Gly can be replaced by apolaric amino acids with bulky side chain.

Ala-Leu exchange resulted in a more active compound.

There is no selectivity to HT-29 cells.

KK24 shows 2-5 times higher antitumor activity than KK06/2.

Searching the target receptor is in progress.

Conclusion

- Sequence optimization of homing peptides selected by phage display might provide more active Drug Delivery Systems (DDS) for targeted tumor therapy.
- The sequence has influence not only on cellular uptake but also on the serum/plasma stability.
- VHLFYAT sequence based homing peptide might be good candidate for the development of DDSs for a broad spectrum of tumor types.

References

[1] http://[1] http://ec.europa.eu/health/
[2] Y. Zhang., et al. **2007** *J. Biomol. Screen.* 12, 429-435 .

Acknowledgments

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