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CATHEPSIN INHIBITION BY SMALL PEPTIDES

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ABSRACT

Background: Previous paper results indicate that small peptides can be found from DNA translation. Translated peptides can be tested against enzymes like cathepsin having both positive and negative effect on cancer cells. **Material and methods:** DNA reaction and organic synthesis of DNAs that translate small peptides, cell viability assay etc. were performed. **Results:** 3 novel peptides found which inhibits Cathepsin enzyme in MDA-MB-231 and MCF-07 breast cancer cells.

KEYWORDS: MCF-07, MDA-MB-231, Breast cancer.

INTRODUCTION

DNA library require short stretches of DNA sequences. In this work, we report a novel technology[1-6] for block shuffling of DNA. 8 DNAs 7 times in cyclic method elongated by shuffling. Then 2 major oligonucleotides used to produce DNAs that inhibit cathepsin enzyme.

MATERIALS AND METHODS

The following DNAs were manufactured from alphadna, Canada.

GGCTCGCGAATACTGCGAAGACCACCATGGGC
GGCTCGCGAATACTGCGAAGACCACCATGATC
GGCTCGCGAATACTGCGAAGACCACCATGGAC
GGCTCGCGAATACTGCGAAGACCACCATGAAG
GGCTCGCGAATACTGCGAAGACCACCATGTCC
GGCTCGCGAATACTGCGAAGACCACCATGTGC
GGCTCGCGAATACTGCGAAGACCACCATGCCA
GGCGATCTCACTCCTTCGCAGTATTCGCGAGCC
ATCGATCTCACTCCTTCGCAGTATTCGCGAGCC
GACGATCTCACTCCTTCGCAGTATTCGCGAGCC
AAGGATCTCACTCCTTCGCAGTATTCGCGAGCC
TCCGATCTCACTCCTTCGCAGTATTCGCGAGCC
TGCGATCTCACTCCTTCGCAGTATTCGCGAGCC
CCAGATCTCACTCCTTCGCAGTATTCGCGAGCC
GGCTCGCGAATACTGCGAAGACCACCATG
GGCTCGCGAATACTGCGAAGGAGTGAGATC
CTGCGAAGACCAC
GCGAAGGAGTGA

The followings were purchased-
Biotin and phosphorylation kit SA magnetic beads MCF-07, MDA-MB-231 Breast cancer cells

The followings were done---
PEG suspension preparation
Primer mixture preparation
Tris preparation
Magnesium chloride preparation
BSA preparation
Magnet collection
Sodium bicarbonate preparation
NAP5 column preparation
PPTIFFRLK preparation
EMCS (N--malemidocaproyl-oxysuccinimide ester) preparation
EDTA preparation
Tween-20 preparation
Acetonitrile preparation
phosphate buffer preparation
Procedure
Ligation
9 oligonucleotides were ligated
Restriction endonuclease
The DNAs were cut with enzyme

Biotinylation

The DNAs were attached to biotin

Phosphorylation

Phosphate group was added to DNAs
Polyacrylamide electrophoresis of primers
The electrophoresis was done but was unsuccessful

The above peptide was custom manufactured EMCS (N--malemidocaproyl-oxysuccinimide ester) preparation-EMCS and 2 kinds of final single stranded DNA was

kept at room temperature for 1 hour the mixture was precipitated from acetonitrile by solvent evaporation
M phosphate buffer added
NAP5 column

The column desalted preparation
Solvent evaporation overnight incubation with peptide the preparation was incubated overnight NAP5 column

Again the column desalted magnet treatment the preparation was magnet treated ammonia buffer treatment the preparation was alkalinized with ammonia water puromycin was attached to the DNAs peptides were added to human breast cancer cells after sequencing the peptides were found to inhibit cathepsin enzyme

RESULTS

The following 3 peptides inhibited cathepsin enzyme ----
-
IEGRVGCDFMYVG
KGPPPCPC
VGCDFMYV

CONCLUSION

The peptides are being recommended for therapeutic dose preparation.
ConFliCt of inteRests: The Authors declare that they have no conflict of interests.

AuthoRs' ContRibutions
Nishat Chowdhury, Dhanasekaran Ganeshan did all experiments. Nishat Chowdhury financed the entire experiment and Chinnasamy Arulvasu provided his laboratory for use. Shingo Ueno and Yuki Mochizuki designed the study.

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