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13. ABSTRACT (Maximum 200 Words)

The annual report for postdoctoral traineeship awards "Optimization and Characterization of Prostate Cancer Targeting Peptides" covers the period 02/01/2003-01/31/2004. During the first year, the combinatorial libraries proposed in specific aim 1 were synthesized and screened for DU-145, LNCap, PC3 cell binding (specific aim 2). The libraries were tested for binding of other cancer cell lines such as ovarian, lung and pancreatic cancer cell lines. Several high affinity ligands were identified for ovarian and lung cancer cell lines, but only weak binding ligands for prostate carcinoma cell lines were identified. The following structure activity experiments (specific aim 3) were performed with ligands for DU-145 found previously and resulted in identification of essential amino acids. Furthermore, this ligand has been found to be specific for the prostate carcinoma cell line DU-145. Based on the results of structure activity study the next generation of combinatorial libraries are being synthesized.

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Introduction

In this project, we plan to characterize and further optimize the previously identified peptide ligands for cell surface receptors expressed on the DU-145 prostate carcinoma cell line. The newly identified ligands will eventually be developed as reagents for photoaffinity labeling and to isolate cell surface receptors unique for DU-145 cell line.

Body

During the first year of funding period, the "one-bead one-compound" combinatorial libraries proposed in **Task 1a** were synthesized. Each library contained 3,111,696 compounds and the density of the ligand on the exposed surface was 100% for the first library and 20% of maximal solid support substitution for second library. The reason for "20% down-substituted" library synthesis was to identify the ligands with strong affinity to tested cells that could bind to the beads even if the ligand concentration on the bead surface was lower. The general structure of the libraries is $cX^1GX^2GX^3X^4c$. The building blocks were chosen according to the consensus found in the previously identified peptide ligands. The secondary structure of the previously identified ligands was determined by NMR and molecular modeling study. Particularly, for X^1 42 natural and unnatural amino acids were used bearing active hydrogen that is capable of participating in hydrogen bond interactions. For X^2, X^3 and X^4, 42 bulky and hydrophobic unnatural amino acids were used. Based on the NMR secondary structure study and molecular modeling experiments, we proposed that there would be a general conformation similarity among the cyclic octapeptides with cXGXGXXc motif. An overlay of five proposed secondary structures is shown on Fig 1 and the β-turn in N-terminal part of the molecule involving first four amino acids cX^1GX^2 (Fig 1) has been found in all NMR analyzed samples.

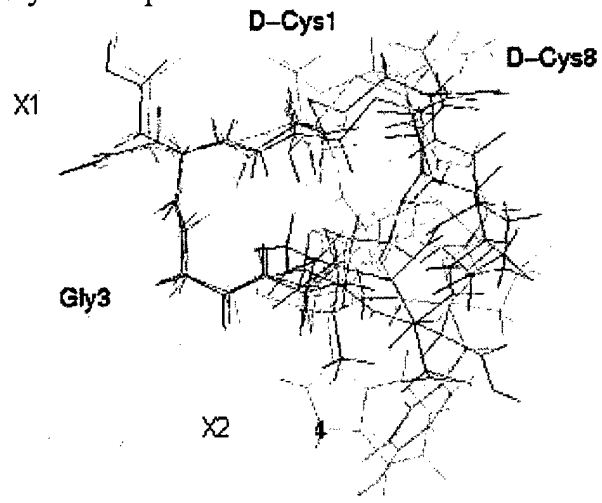

Fig.1 The proposed secondary structure of five compounds with $cX^1GX^2GX^3X^4c$ motif.

Both libraries were tested for binding of DU-145, LNCap, PC3 prostate carcinoma cell **(Task 1b)**, as well other cancer cell lines such as ovarian, lung and pancreatic cancer cell lines **(Task 4a)**. Several high affinity ligand were identified for ovarian cancer cell lines ES-2 and SKOV-3, but we were unable to identify novel high affinity ligands for prostate carcinoma cell lines.

The following structure activity experiments **(Task 3a)** were performed with D-amino acid composed ligand (kikmviswkg) for DU-145 found previously [1]. The alanine walk study was conducted, where one amino acid in the parent sequence is replaced with alanine and the activity is compared to the parent peptide, was used to find the amino acids essential for the interaction with cell surface receptors. The peptides were synthesized with covalently bound biotin and their affinity to DU-145 cells was evaluated by flow cytometry **(Task 3b)** using fluorescently labeled streptavidin as the fluorescent label. The results of alanine walk experiments are summarized in Chart 1. The fluorescent labeling activity of alanine walk peptides is compared to the parent peptide (left bar) and the altered amino acid is indicated on x-axis. The results suggest that the peptide interacts with cell surface receptors using the hydrophilic amino acids in position 1,3,5,7 and 9. The amino acids in other positions were not significant for the interaction. We anticipate that the substitution of these amino acids with other unnatural building blocks can modulate and eventually improve the affinity of future ligands.

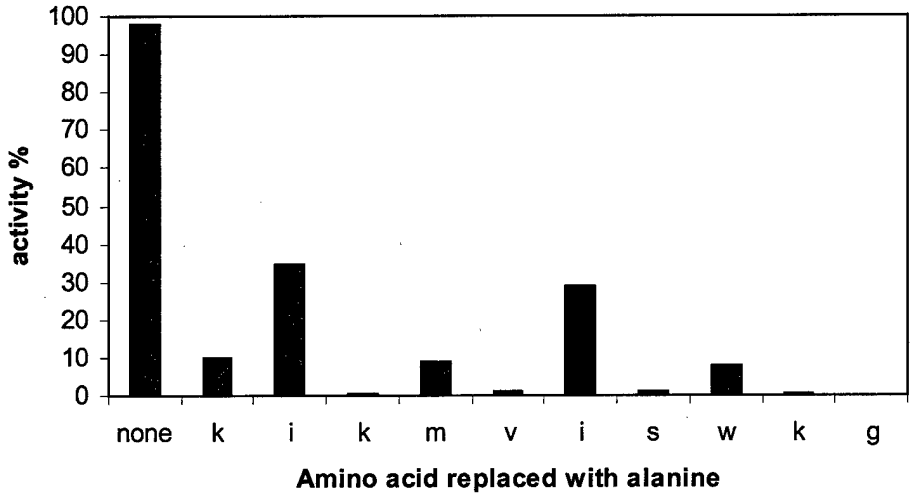

Chart 1. Alanine walk experiment results. The activity of peptides with amino acid replaced with alanine is compared to activity of parent decapaptide kikmviswkg (leftmost bar).

The truncated parent peptide analogs were found to be inactive suggesting that the decapeptide is the shortest peptide capable the interaction with DU-145 cells. Based on the results of structure activity study, the next generation of combinatorial libraries are being synthesized.

The parent peptide kikmviwkg was tested for interaction with two non-carcinoma prostate cell lines Prostate Stroma Cell (PrSC) and Prostate Epithelial Cell (PrEC) in order to determine its specificity to carcinoma cell line DU-145. The staining level of the PrSC and PrEC with biotin-peptide conjugate and flourescently labeled streptavidine was determined by flow cytometry. The level of staining for both non-carcima cell lines was 30% compared to the DU-145 (Chart 2), which indicates that the receptor for the peptide is present in healthy prostate tissue but the level of the expression of the receptor is significantly higher in carcinoma cell line DU-145.

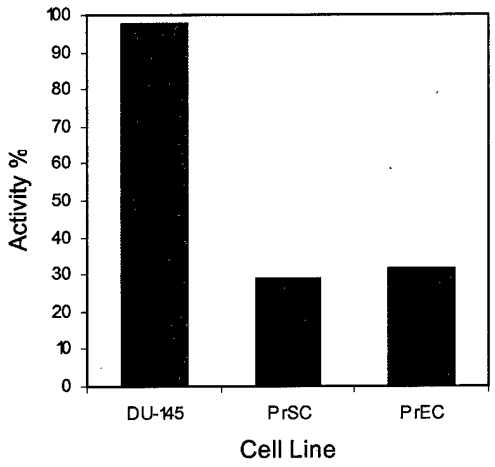

Chart 2. Staining of DU-145 and non-carcinoma prostate cell lines PrSC and PrEC with kikmviswkg-biotin conjugate.

Key Research Accomplishments

- **Task 1a** Two combinatorial cyclic peptide libraries were synthesized with different ligand density on the exposed surface. The design of libraries was based on NMR study and molecular modeling experiment performed with previously identified peptides.
- **Task 1b** Libraries from task 1a were screened for cell binding of prostate carcinoma cell lines DU-145 , LNCap and PC3 but only weakly bindings ligands were identified.
- **Task 4a** Libraries from task 1a were screened with other cancer cell lines (ovarian, prostate, lymphoma, lung) and ligands with high affinity towards ovarian carcinomas ES-2 and SKOV-3 were identified.
- **Task 3a** Structure activity experiments were performed with (kikmviswkg) peptide identified previously in our lab [1]. Two sets of biotinylated peptides were designed, the alanine walk experiment (10 peptides) and truncated peptides (10 peptides) were prepared.
- **Task 3b** Peptides from task 3a were tested for cell staining using flow cytometry and fluorescently labeled streptavidin. The amino acids essential for interaction of the peptide with cell surface receptors were identified.
- **Task.4a** The peptide kikmviswkg was tested with the non-carcinoma prostate cell line lines PrEC and PrSC and compared to its affinity to the carcinoma cell line DU-145. Specificity to DU-145 was determined.

Reportable Outcomes

Aina, O. H.; Marik, J. and Lam, K. S. Identification of Novel Peptide Ligands for Ovarian Adenocarcinoma. In 6th Joint AACR/JCA Conference, *Advances in Cancer Research*, Waikoloa Village,HI January 25-29 2004.

Conclusion

Two more than three million members combinatorial cyclic peptide libraries containing mostly unnatural amino acids were synthesized and screened for cell binding to the DU-145 cell line and other carcinoma cell lines. Libraries based on ligands with cXGXGXXc motif have not provided good ligand leads for DU-145 cell line, but the high affinity ligands for ovarian cancer cell lines have been identified. We next focused our efforts on optimizing the binding of the decapeptide, kikmviswkg, to prostate cancer cells by performing structure activity study. This study provided important information for the ongoing preparation of second generation focused libraries. The experiments with non-carcinoma prostate cell lines PrSC and PrEC showed the specificity of the decapeptide to carcinoma cell line DU-145.

References

1. DeRoock, I. B.; Pennington, M. E.; Sroka, T., Lam, K. S.; Bowden, T. G.; Bair, E. L. and Cress, A. Synthetic Peptides Inhibit Adhesion of Human Tumor Cells to Extracellular Matrix. *Cancer Research* 2001, **61**, 3308-3313.

Appendices

No appendices.