Discovery of an Apoptosis Inducing Ligand for Burkitt Lymphoma

Carolyn Laymon[1], Kyla Bradylong[2], Mary Saunders[3], David Olivos[3], and Kit Lam[3]

[1]School of Education, California State University, Sacramento, [2]Department of Biological Sciences, California Polytechnic State University, San Luis Obispo, [3]Department of Biochemistry and Molecular Medicine, University of California Davis, School of Medicine

Abstract

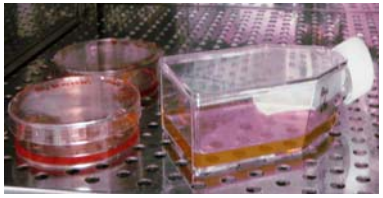
One-bead two-compound (OBTC) combinatorial chemistry libraries enable the discovery of novel synthetic compounds which can be used to evoke specific signaling responses in cells. The library configuration is composed of a fixed known cell adhesion ligand and a random chemical library displayed on the surface of Tentagel beads. The cell adhesion ligand binds to specific receptors located on the surface of cells enabling the random immobilized chemical molecules on each bead resin to evoke specific cellular responses such as apoptosis or cell death. To validate this concept, a OBTC combinatorial library comprised of an α4β1 integrin targeting ligand, LLP2A, and a novel self-folding tricyclic branched hexamer random library were screened against various hematological and epithelial cancer cell lines: Raji, Molt4, Jurkat, TK6, and PC3N. These cells were incubated with library beads for 48 hours in 6 well tissue culture plates. Propidium iodide, a DNA intercalating agent, is then added to each well to evaluate cell viability. When visualized under a fluorescent microscope, with wavelength excited at 488 nm, cells bound to the OBTC libraries will fluoresce red, indicating apoptosis. From the Raji cell line screening, one bead from the LDO2A-LLP2A library was selected for invoking apoptosis. The morphological appearance of the cells bound to this bead were: blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. Further sequencing via Edman degradation will be performed to identify the amino acid sequence. This chemical approach has the potential to target and kill Burkitt lymphoma cancer cells.

Background

Burkitt lymphoma is an aggressive B-cell non-Hodgkin lymphoma that occurs most often in children and young adults. The disease may affect the jaw, central nervous system, bowel, kidneys, ovaries, or other organs. There are three main types of Burkitt lymphoma (sporadic, endemic, and immunodeficiency related). Sporadic Burkitt lymphoma occurs throughout the world, and endemic Burkitt lymphoma occurs in Africa. Immunodeficiency-related Burkitt lymphoma is most often seen in AIDS patients. Novel chemical compounds that target and specifically kill these fast growing cancer cells are needed to improve current treatments and prevent tumor lysis syndrome from chemotherapy.

Materials and Methods

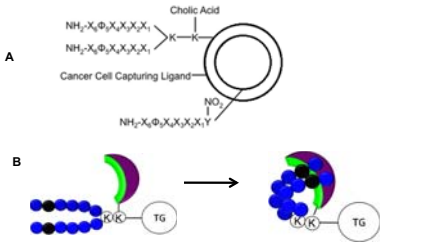
Cell culture



Hematological and epithelial cancer cell lines (Raji, Molt4, Jurkat, TK6, and PC3N) were cultured in ATCC-formulated RPMI-1640 and F-12 medium,10% fetal bovine serum (FBS), 100 U/mL penicillin G, and 100 µg/mL streptomycin at 37°C using a humidified 5% CO2 incubator

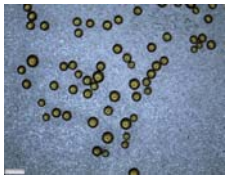
OBTC combinatorial library

Combinatorial library beads

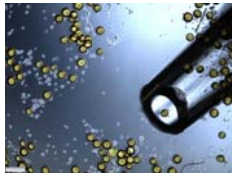


(A) Schematic representation of a novel self-folding tricyclic branched hexamer protein mimic combinatorial library with a cancer cell capturing ligand for OB2C screening. The branched hexamer chains serve as random chemical motifs to screen for cell killing activity. (B) Cartoon schematics of the tricyclic library (unfolded and folded, respectively).

Cell screening with library beads

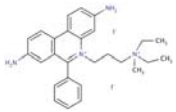


Library beads with cells (4X)



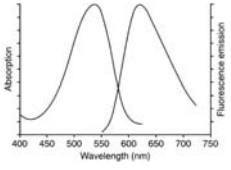
Selecting positive beads using a micropipetter (4X)

Identification of apoptotic ligands with propidium iodide (PI)



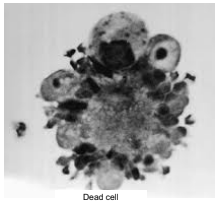
Structure of PI Molecular mass: 668.4 Da

PI binds to DNA by intercalating between the bases with one dye per 4-5 base pairs of DNA



Fluorescence excitation at 488 nm and emission profiles of PI bound to dsDNA

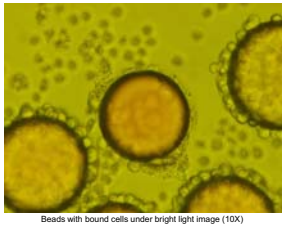
Apoptosis



Dead cell

Morphological Changes

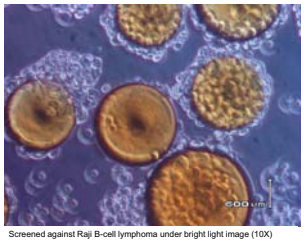
- Refraction of cell body
- Formation of membrane blebs
- Disintegration of nucleus
- Formation of apoptotic bodies



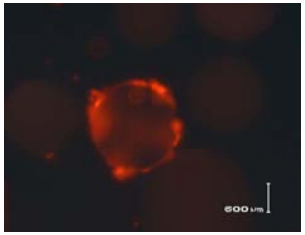
Beads with bound cells under bright light image (10X)

Bead with bound cells under fluorescent microscope (10X)

Results



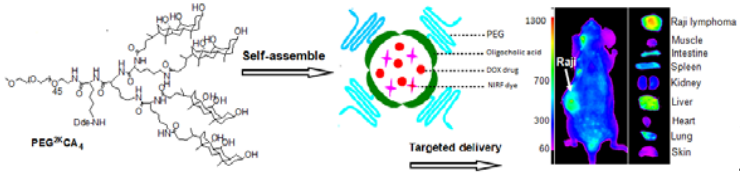
Screened against Raji B-cell lymphoma under bright light image (10X)



Screened against Raji B-cell lymphoma under fluorescent microscope (10X)

- ❖OBTC combinatorial library comprised of an α4β1 integrin targeting ligand, LLP2A, and a novel self-folding tricyclic branched hexamer random library were screened against Raji B cell lymphoma for proapoptotic signaling
- ❖After 48 hours incubation with library beads, propidium iodide was added to identify OBTC beads inititating cell death
- ❖Despite 25 million beads binding to the various cancer cells, only one bead displaying fluorescently labeled dead cells is isolated for sequence analysis via Edman Degradation

Future Research



- ❖Apply the OBTC approach to multi-functional nanoparticles developed in the Lam laboratory
- ❖Alpha 4 beta 1 integrin targeting ligand
- ❖Discovered apoptosis inducing ligand for Burkitt lymphoma

GOALS

1) Selective delivery of cytotoxic agents to tumor mass while sparing normal organs
2) Enhance anti-tumor effect
2) Lessen the systemic side-effects of drugs
3) Improve therapeutic index and efficacy

Acknowledgements

This material is based upon work supported by the S.D. Bechtel, Jr. Foundation and by the National Science Foundation under Grant No. 0952013 and Grant No. 0733758. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the S.D. Bechtel, Jr. Foundation or the National Science Foundation.