FMRI RET 2014-Purification of a Fusion Protein for the Treatment of Cancer Cells

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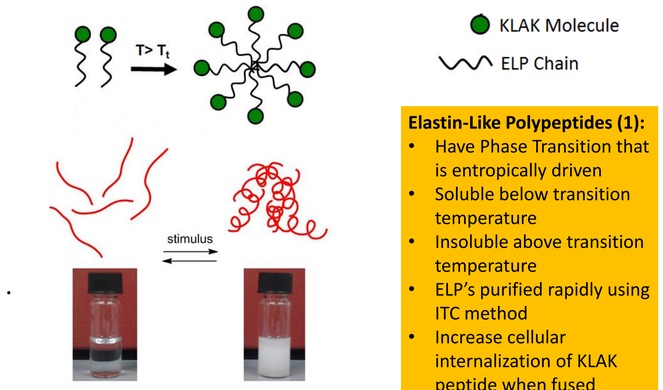
Abstract

$(KLAKLAK)_2$ is a cationic α-helix peptide that is well known to cause cell death once it is internalized by cells Previous work have shown that internalization of $(KLAKLAK)_2$ is facilitated when it is part of a nanostructure. In this work, we purified $(KLAKLAK)_2$ fused to elastin-like polypeptides (ELPs). ELPs are protein-based polymers which are thermoresponsive polymers and whose solubility changes at a particular temperature called transition temperature. It is this transition temperature that allows for the purification of the fusion $(KLAKLAK)_2$-ELP using the well documented method of inverse transition cycling (ITC). In addition, the ELP backbone allows for $(KLAKLAK)_2$ to be part of a nanostructure which improves its internalization. After the purification of the $(KLAKLAK)_2$-ELP, lung cancer cells (A549) were treated with the fusion and a live/dead assay was performed to test its cytotoxic effect. The results obtained clearly showed that $(KLAKLAK)_2$-ELP causes cell death at high concentrations.

Elastin-Like Polypeptides



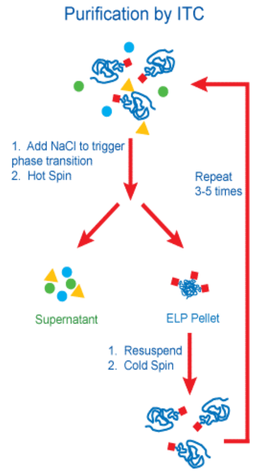
Reference: http://www.ru.nl/bio-orgchem/research/research_projects/mark-van-eldijk/

Elastin-Like Polypeptides (1):

- Have Phase Transition that is entropically driven
- Soluble below transition temperature
- Insoluble above transition temperature
- ELP's purified rapidly using ITC method
- Increase cellular internalization of KLAK peptide when fused

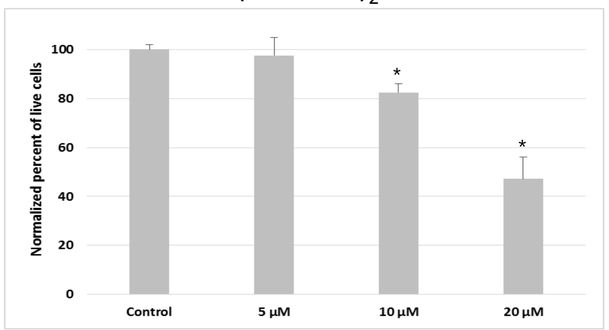
Methods

After ligation/transformation of the fusion protein $(KLAKLAK)_2$-ELP into E.Coli, the bacteria were cultured and lysed. The fusion protein was the purified using the Inverse Transition Cycle (ITC) method. Concentrations of 5 µM, 10 µM, and 20 µM were prepared in DMEM + 0.5% FBS + 1% AA. Lung carcinoma cells A549 were seeded at 7×10^3 cells/well in 12 wells of a 48 well plate. The wells were treated (3 wells per concentration) with respective concentrations of prepared solutions, the control was treated with media only. After 96 hours of treatment fluorescent microscopy pictures were taken and analyzed using image J.



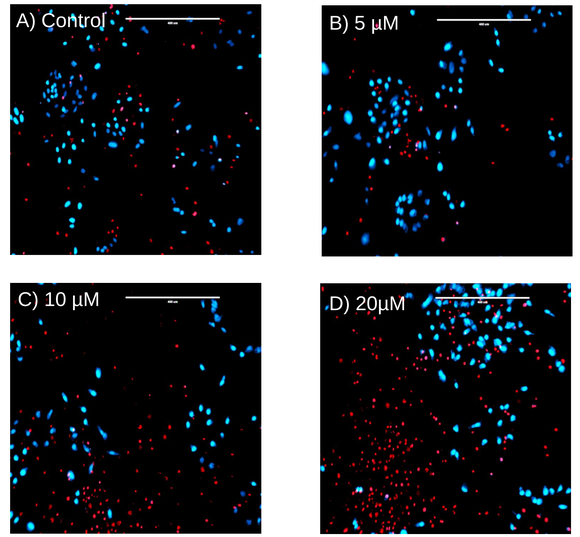
Live/Dead Assay Results

High Concentrations of $(KLAKLAK)_2$-ELP Causes Cell Death



Cell Death at Increasing Concentrations of Fusion Protein: 7×10^3 cells/well were seeded in 12 wells of a 48 well plate, serum starved, and treated for a 96 hour period with scaled concentrations of KLAK-ELP protein (3 wells at respective concentrations). The control was treated with serum only. A live/dead assay was then performed using fluorescent microscopy and Image J. Result show a linear trend with 20 µM concentration the fused lytic peptide killing up to 58% of cells.
* denotes significant results based on p-value

Fluorescent Microscopy of A549 Cell Death



Fluorescent Microscopy Images of Cell Death at Scaled Concentrations of Lytic Peptide: A) Control, B) 5 µM, C) 10 µM, D) 20 µM. Blue fluorescence indicates live cells and red fluorescence indicates dead cells. Results show cell death increases with concentration.

Conclusions

- At higher concentrations of KLAK-ELP cellular death of lung cancer cells A549 increased significantly
- The use of KLAK-ELP has promising indications for future treatment of carcinogenic cells and should further be investigated.

Referenced Resources and Acknowledgments

1) Piyush Koria, Hiroshi Yagi, Yuko Kitagawa, Zaki Megeed, Yaakov Nahmias, Robert Sheridan, and Martin L. Yarmush. "Self-assembling elastin-like peptides growth factor chimeric nanoparticles for the treatment of chronic wounds.", PNAS 2011, 108, 1034-1039

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