CALIFORNIA SCIENCE & ENGINEERING FAIR
2018 PROJECT SUMMARY

Name(s)

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Project Number

38039

Project Title

THY-1 Antibody Fragment Dye Contrast Agents for Novel Pancreatic Cancer Detection

Abstract

Objectives/Goals

The objective of this study was to synthesize a new contrast agent probe for imaging of pancreatic cancer.

Methods/Materials

10 micrograms of THY-1 single chain antibody variable fragments (scFv) were reduced with tris(2-carboxyethyl)phosphine to prevent dimerization. The reduced THY-1 sample was then incubated with MAL-PEG4-NH2, the PEG crosslinker, to prep for bioconjugation. This sample was then bioconjugated with a 1:1 molar ratio of indocyanine green. 10 micrograms of THY-1 antibody was directly bioconjugated to Indocyanine Green dye in varying molar ratios to find the ideal molar ratio for synthesis. Bioconjugation was validated with mass spectrometry and spectrophotometry. Binding assay tests were conducted to test affinity and effectiveness using a Guava EasyCyte Flow Cytometer with beads and cells (in vitro) coated with the THY-1 antigen.

Results

The binding performance of the THY-1 scFv conjugate contrast agent strongly outperformed that antibody dye conjugate contrast agent. The ideal THY-1 scFv dye conjugate dye-protein ratio was found to be 100:1, with a binding ratio of 68.55%; the 100:1 THY-1 antibody dye conjugate had a binding proportion of 22.46%. This 100:1 THY-1 scFv dye conjugate outperformed the 100:1 antibody-dye conjugate.

Further, mass spectrometry found that the 100:1 reactions yielded 2:1 dye:protein ratios in practice, with the spectrophotometry results implying that a 7-8 dyes can bind to a single protein because of binding sites.

Conclusions/Discussion

A new THY-1 scFv dye conjugate was synthesized for pancreatic cancer imaging. These new Thy-1 scFv dye conjugates have stronger binding affinity that their full antibody conjugate counterparts, implying that scFv-dye conjugates are more ideal despite their smaller size. Smaller size and better clearance properties thus make this newly synthesized probe ideal for mass screenings of pancreatic cancer.

Summary Statement

I designed and synthesized a new antibody fragment dye conjugate for pancreatic cancer diagnosis with better accuracy and lower biotoxicity than current diagnostic methods.

Help Received

Dr. Juergen Willmann (Stanford) mentored and vetted my work. Dr. Katheryne Wilson (Stanford) and Dr. Lotfi Abou El-Kacem (Stanford) mentored me and taught me critical lab techniques. Mr. Ken Lau (Stanford) operated the MALDI-TOF mass spectrometer.