

Sputum-Based mRNA-Targeting Probes in Lung Cancer - An Early Diagnostic Tool

Project ID: #1918, Category: CB - Computational Biology and Bioinformatics, Division: Senior, Science Type Project

Research Question: Can a sputum-based multiplex RNA probe targeting mRNA sequences of various tumor marker genes that are well-known for their over-expression in lung cancer be developed?

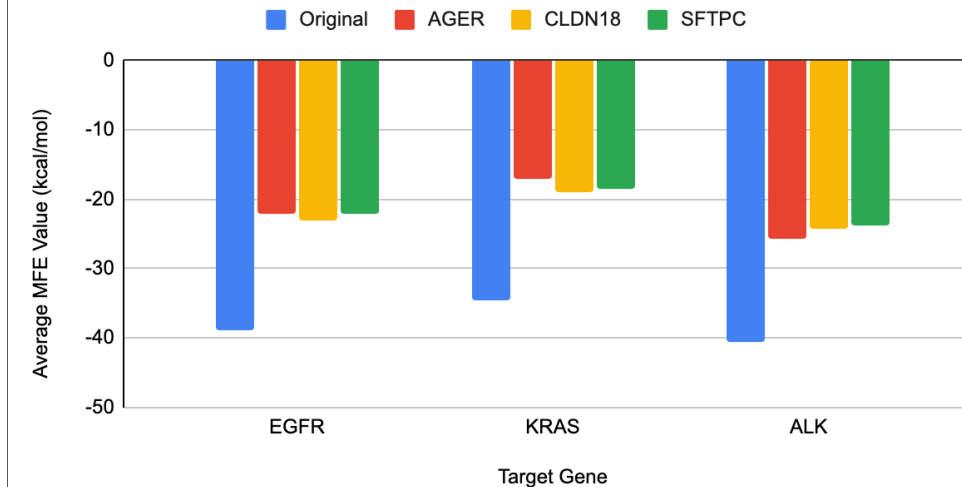
- Lung cancer is the leading cause of cancer death
- Noninvasive probes for early detection will likely reduce mortality
- mRNA is a promising target, as being single-stranded allows probes to easily bind to it, and lipid vesicles shed by lung cancer keep it from degrading in sputum

Methodology and Data Analysis

- mRNA sequences for EGFR, KRAS, and ALK target genes were folded into secondary structures
- Open regions (21 nucleotides long) from these secondary structures were recorded 6 times per target gene (18 sequences total)
- Reverse complement of each sequence found (to generate complementary probes)
- RNAhybrid (Linux tool) was used to calculate the minimum free energy (MFE) of the hybridization, which indicates stronger hybridizations as the value becomes more negative, of each probe to its target
- The 3 probes with the most negative MFE values for each gene were chosen with results reconfirmed using the DuplexFold Web Server
- Chosen probes were also tested against AGER, CLDN18, SFTPC (control genes), all of which are lung-specific

Results

Average MFE Values (kcal/mol) for the 3 Chosen Probes for Each Target Gene in Comparison to Those of the Control Genes



MFE values are more negative for the hybridization with the target genes than with the control genes

Interpretation & Conclusion

- The generated probes are effective and target-specific, as they hybridize more strongly to their target mRNA than the control mRNA
- Clinical testing on sputum from lung cancer patients should later be done to verify the efficacy of the probes compared to sputum from healthy individuals
- I plan to conduct this pilot trial and partner with a manufacturer to produce the multiplex probe (utilizing all of the 9 sequences)
- I also plan to use this methodology to make noninvasive detection tests for other types of cancer in urine, feces and saliva