Circulating Cell-Free DNA in the Diagnosis of Cholangiocarcinoma

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Introduction

- The aim of the project was to investigate the diagnostic value of circulating cell-free DNA (cfDNA) for Cholangiocarcinoma (CCA).
- CCA forms in the bile ducts inside the liver and is often diagnosed in an advanced stage.
- cfDNA is an extracellular DNA that is released into the bloodstream by necrotic/apotoptic cells.
- In cancer patients, increased levels of cfDNA in the blood are frequently observed [1].
- Despite there being much ongoing research focusing on the profiling of circulating tumor DNA (ctDNA), these analyses are not yet established in the clinic and are currently expensive [2].

Materials and Methods

- Data: DNA data from tumor tissue and cfDNA from 8 CCA patients with stage I($n = 1$), II($n = 3$) and IV($n = 4$) was analyzed. 60 mutated genes previously reported with high frequency in CCA were targeted for analysis using the reference human genome GrCh38.
- Sequencing: Illumina NovaSeq 6000 with targeted capture strategy, 150 bp paired-end reads.

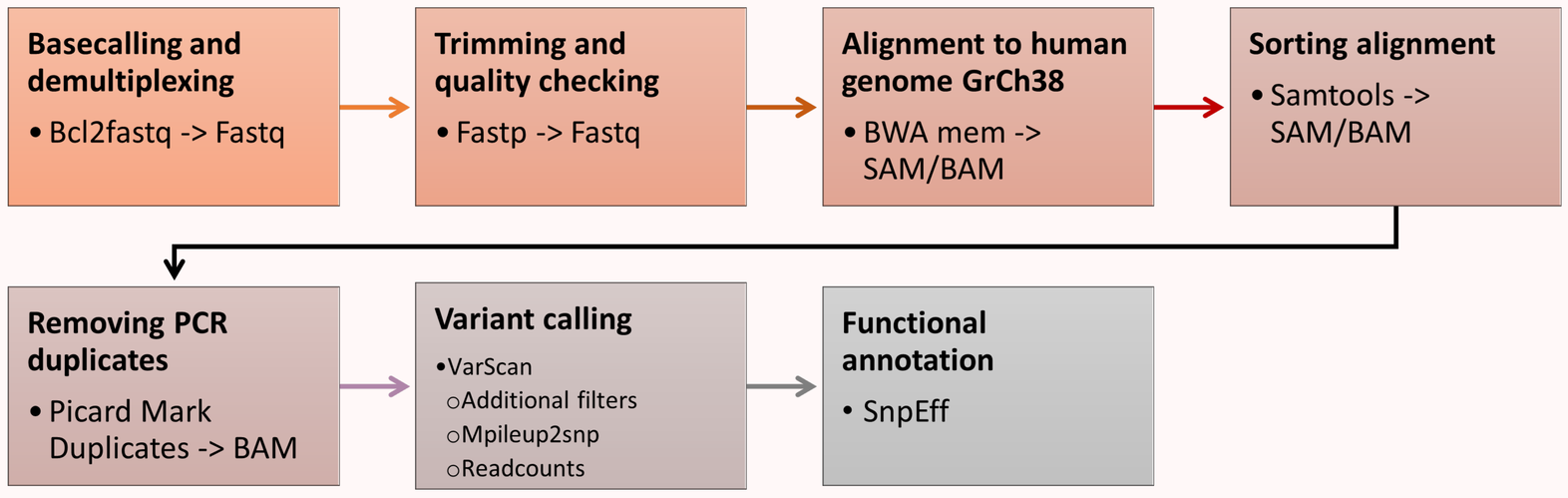


Figure 1 The workflow for the NGS analysis.

Discussion and Conclusion

- Circulating cfDNA could potentially serve as an important non-invasive diagnostic tool for cancer as the mutations detected in the cfDNA are relatively representative of the CCA tumor tissue.
- Variations in insert sizes between the stages of cancer was detected in the cfDNA. The highest stages of CCA had the shortest insert sizes.
- However, the data analysis was performed on a limited sample size and further investigation is required to support the findings.

Future Perspectives

- Future studies could be conducted to compare the levels of cfDNA between various types of cancer.
- Machine learning strategies, including deep learning, could be employed to understand cfDNA predominantly in early-stage as they contain minimal tumor DNA content for identification.

Results

The cfDNA insert sizes where compared between three patients with stage I, II and IV CCA. It can be seen in figure 2 that the insert sizes correlate with the progression of CCA in patients. Thus, shorter cfDNA insert sizes indicate a higher cancer stage in the patient.

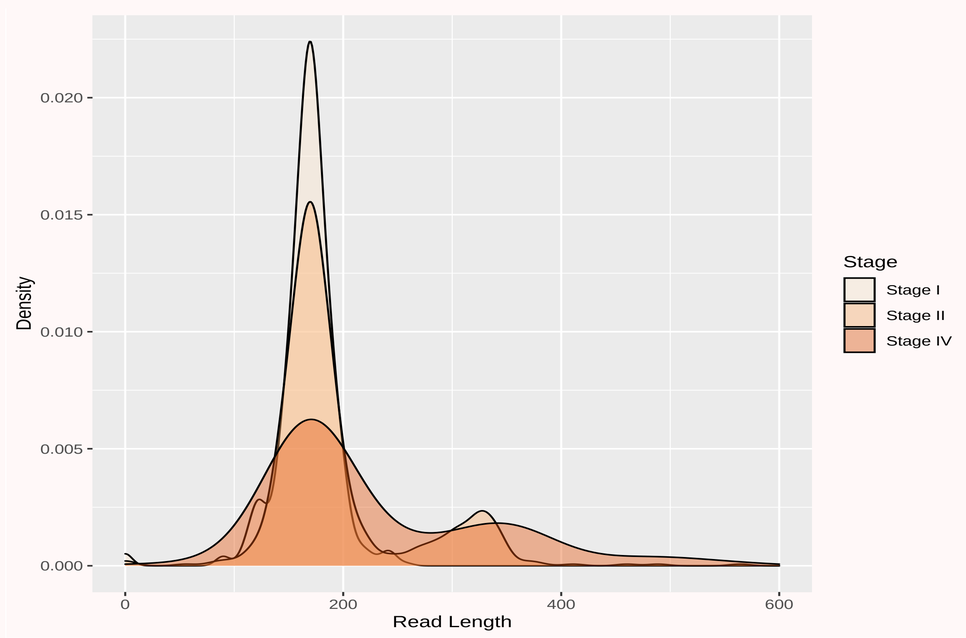
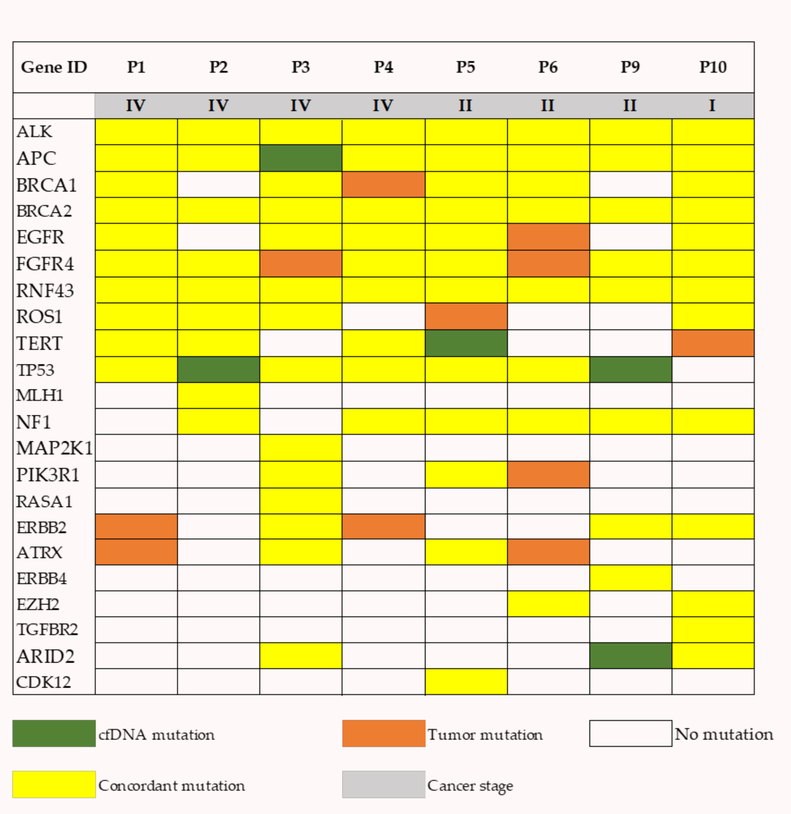


Figure 2. Insert size distribution of cfDNA.

Figure 3, left, shows a heatmap of mutation occurrences. The heatmap was constructed by detecting the missense variants found in the cfDNA and consists of 22 genes. Concordant mutations are those detected in both tumor tissue and the cfDNA. It can be seen that there are many concordant mutations in all the patients which suggests that one can detect mutation occurrences in the cfDNA. The percentage of concordant mutations was 46.6% while mutations found only in cfDNA were 0.28% and 6.3% for tumor tissue mutations.

The variant allele frequency (VAF) was computed for the tumor tissue DNA in which the vcf files included all variant sides. As was expected, the mean VAF for stages II and IV was the highest, however the mean for stage II was higher than for stage IV, see figure 3 (right). Furthermore, it can be seen that the distribution spans a wider range for the higher stages.



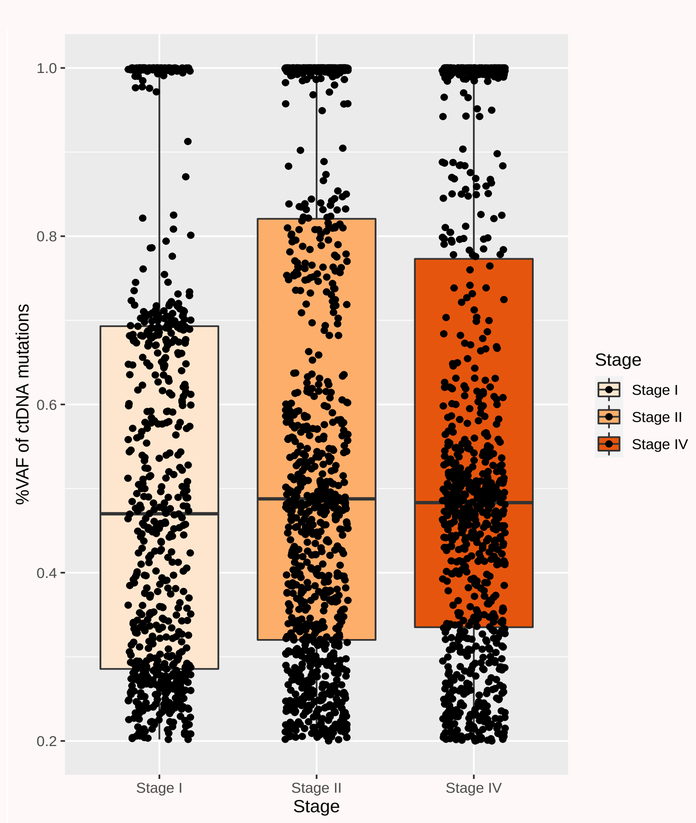


Figure 3. Left: A heatmap showing the mutation occurrences in cfDNA compared to CCA tumor tissue. **Right**: A boxplot of VAF between the three stages of cancer, I($n = 1$), II($n = 3$), IV($n = 4$).

References

[1] P. Wintachai et al., *Diagnostic and Prognostic Value of Circulating Cell-Free DNA for Cholangiocarcinoma*, Diagnostics, vol. 11, no. 6, p. 999, May 2021, doi: 10.3390/diagnostics11060999.

[2] D. Fernandez-Garcia et al., *Plasma cell-free DNA (cfDNA) as a predictive and prognostic marker in patients with metastatic breast cancer*, Breast Cancer Res., vol. 21, no. 1, p. 149, Dec. 2019, doi: 10.1186/s13058-019-1235-8.