Harnessing aqueous humor circulating tumor dna for retinoblastoma stratification

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Abstract

Background

Retinoblastoma (RB) is the most common intraocular malignancy in children and presents significant diagnostic challenges due to its critical location within the eye and the high risk of tumor seeding, which precludes traditional tissue biopsies. There is a critical need for non-invasive molecular risk stratification prior to enucleation.

Objective

This study investigates the use of aqueous humor (AH) as a minimally invasive source for liquid biopsy, aiming to evaluate its potential for molecular profiling of circulating tumor DNA (ctDNA) in RB patients and establish it as a tool for pre-enucleation molecular risk stratification.

Methods

We analyzed AH-derived ctDNA from 19 enucleated eyes of RB patients using low-coverage whole-genome sequencing (lcWGS) and methylation profiling. These data were compared with matched tumor tissue DNA to assess genomic alterations and methylation patterns. A random forest classifier was trained on ctDNA methylation data to classify tumors into distinct molecular groups. Additionally, copy number variations (CNVs) were examined in both AH ctDNA and tumor tissue DNA to evaluate the sensitivity of lcWGS versus methylation arrays.

Results

The analysis revealed a high concordance between AH ctDNA and tumor tissue DNA for both genomic alterations and methylation patterns. A random forest classifier trained on methylation data achieved an area under the curve (AUC) of 1.0, accurately classifying tumors into distinct methylation clusters. CNVs were reliably detected in both AH ctDNA and tumor tissue DNA, with lcWGS outperforming methylation arrays in sensitivity. While some samples showed insufficient cfDNA quality, the majority (90%) provided robust results.

Conclusion

This study demonstrates that AH-derived ctDNA is a reliable and effective method for molecular tumor classification in RB. The high concordance between AH ctDNA and tumor tissue DNA, along with its ability to predict methylation subgroups and detect CNVs, highlights the potential of AH liquid biopsy for non-invasive early diagnostics. This approach could revolutionize RB care by overcoming the limitations of traditional biopsies, advancing precision medicine, and improving risk stratification.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.