Introduction

Aristolochic acids (AA) are a potent class of mutagen that can be found in plants of the genera Aristolochia and Asarum. Exposures to AA are caused by intentional ingestion of traditional herbal medicines containing Aristolochia or through inadvertent consumption from contaminated crops. The carcinogenic potential and epidemiologic connection of AA exposure with upper-tract urothelial cancers (UTUC), liver cancer, and kidney failure have been known for years. Whole exome sequencing has revealed high levels of somatic mutations in the tissues and liquid biopsies of cancer patients with known AA-exposure. This pattern is highly specific and indicative of AA-exposure and has been cataloged through liquid biopsy (urine sediment or blood) that could also be attributed to AA.

We developed a Duplex Sequencing based assay to test whether error-corrected NGS is capable of detecting rare somatic mutations in the normal non-neoplastic tissues of patients that were confirmed to have AA exposure through either whole genome sequencing (WGS) or whole exome sequencing (WES) of tumors elsewhere. We sought to predictively identify mutational patterns in those patients through liquid biopsy (urine sediments or blood) that could also be attributed to AA.

NGS Accuracy is Required to Detect in vivo Mutagenesis Without Prior Clonal Amplification

We employed Duplex Sequencing to the tissues and liquid biopsies of cancer patients with known AA-exposure status. We demonstrate that Duplex Sequencing is a promising approach for the detection of mutagenic signatures caused by environmental carcinogens, and we foresee Duplex Sequencing as being a powerful tool for the detection of mutagenic signatures caused by environmental carcinogens.

Conclusion

Duplex-Sequencing has the potential to identify at-risk members of the public who have been exposed to Aristolochic Acid or other environmental carcinogenic agents:

- A general mutation signature was detected in non-invasively sampled biopsies of AA-exposed patients. This is the first time we are aware that an AA mutation signature has been detected in non-invasively sampled body fluids.
- Duplex-Sequencing measures the life integrated exposure of mutagenic compounds or processes:
  - The mutational signature of spontaneous deamination, which is primarily caused by deamination from methylated cytosines, is characteristic of normal aging or is the case of AA exposure.
  - The blood of patient AB14, ~34% of the mutations were correlated with platinum drug treatment, and, indeed, this patient was later confirmed to have gone through a cisplatin regimen and was unique to this cohort.

Next-Generation Sequencing (NGS)

Single Strand

Duplex Sequencing

TwinStrand Duplex Sequencing™ Technology

A DuplexSeq™ Adapter has:

1. Identical (or relatable) degenerate tags in each strand.


Evidence for AA Exposure is Modulated By Lifestyle

Individuals are exposed to AA through herbal teas containing Aristolochia/Artemisia and plants that have been used for medicinal or culinary purposes, and many of these plants are still used in traditional or modern medicine. As a result, it may be difficult to avoid exposure to AA. Lifestyle factors can influence the risk of developing AA exposure.

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Contact: https://twinstrandbio.com/contact/