TITLE

Investigating the roles of oncogenic extrachromosomal circular DNA (ecDNA) in cholangiocarcinoma

BACKGROUND

Oncogene amplification drives cancer progression and recurrence and oncogene amplified cancers are generally more aggressive with poorer prognosis. Absent in healthy tissue, ecDNA is an exclusive primary location and mechanism for oncogene amplification in cancer cells. Driving high copy number gene amplifications and non-Mendelian genomic adaptation, ecDNA increases intra-tumoral heterogeneity contributing to tumor progression, enabling rapid acquired resistance to targeted therapies and resulting in poor patient outcomes.
Cholangiocarcinoma is an aggressive malignancy with increasing incidence and mortality. It exhibits significant heterogeneity, both in terms of its molecular characteristics and its clinical behavior. This heterogeneity can influence treatment response, prognosis, and disease progression, making it challenging to develop standardized treatment approaches. A deeper understanding of its underlying molecular mechanisms is imperative for the development of more effective targeted therapies.
Despite ecDNA has emerged as a significant player in various cancers, its role in cholangiocarcinoma remains largely unexplored. Memorial Sloan Kettering Cancer Center (MSKCC) has recently implemented the MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) platform, providing a comprehensive picture of the mutational profile and genomic changes of tumors. However, while MSK-IMPACT is highly sensitive in detecting a broad range of genomic alterations, its ability to specifically detect extrachromosomal circular DNA (ecDNA) it's limited. Integrating ecDNA data with MSK-IMPACT results may enhance our understanding of cancer biology and inform personalized treatment approaches.

SPECIFIC AIMS

1. **Characterizing ecDNA prevalence and structure in cholangiocarcinoma specimens.**
   By analyzing a significant number of tumor samples, we anticipate characterizing ecDNA as a prevalent genomic alteration in this cancer type.
2. **Correlating ecDNA profiles with clinical parameters and patient outcomes.**
   Parameters may include tumor stage, histological grade, patient age, and other clinical factors. By investigating the relationship between ecDNA features and patient survival outcomes, we anticipate identifying potential prognostic markers for cholangiocarcinoma. This analysis may reveal specific ecDNA patterns associated with favorable or adverse patient prognosis. Such findings could help clinicians better stratify patients based on their risk profiles.
3. **Integrating ecDNA data with MSK-IMPACT results to identify potential therapeutic targets and biomarkers for cholangiocarcinoma.**
   By identifying genetic alterations present on ecDNA that are associated with treatment response or resistance, we can uncover actionable targets for precision medicine approaches.

METHODOLOGY

- **Study population:** Patients will be eligible if they had a confirmed histologic diagnosis of cholangiocarcinoma, both intrahepatic and extrahepatic. Clinical data will be collected including demographics, overall and disease-free survival, treatments delivered and therapeutic response.
- **Sample collection:** Archival tissue specimens and fresh biopsies from cholangiocarcinoma patients will be obtained from the MSKCC tissue bank. We aim to collect 50 intrahepatic cholangiocarcinoma and 50 extrahepatic cholangiocarcinoma cases, for a total of 100 tumor samples.
- **ecDNA profiling:** Circular DNA sequencing and fluorescence in situ hybridization (FISH) will be employed to characterize ecDNA abundance, structure, and genomic content.
- **Data analysis:** Computational algorithms and bioinformatics tools will be utilized to integrate ecDNA profiles with MSK-IMPACT data. Statistical analyses will be performed to identify correlations between ecDNA features, clinical variables, and genetic alterations.

STUDY TIMELINE

- Protocol evaluation and amendments **11/2024 – 11/2024**
- Sample collection and ecDNA profiling **12/2024 – 03/2025**
- Data analyses ad integration **04/2025 – 06/2025**
- Critical revision of results **07/2025 – 08/2025**
- Manuscript draft production **09/2025 – 10/2025**