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Original contribution

Molecular characterization of gallbladder cancer using somatic mutation profiling☆

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Summary Gallbladder cancer is relatively uncommon, with a high incidence in certain geographic locations, including Latin America, East and South Asia, and Eastern Europe. Molecular characterization of this disease has been limited, and targeted therapy options for advanced disease remain an open area of investigation. In the present study, surgical pathology obtained from resected gallbladder cancer cases (n = 72) was examined for the presence of targetable, somatic mutations. All cases were formalin fixed and paraffin embedded (FFPE). Two approaches were used: (*a*) mass spectroscopy–based profiling for 159 point ("hot spot") mutations in 33 genes commonly involved in solid tumors and (*b*) next-generation sequencing (NGS) platform that examined the complete coding sequence of in 182 cancer-related genes. Fifty-seven cases were analyzed for hot spot mutations; and 15, for NGS. Fourteen hot spot mutations were identified in 9 cases. Of these, *KRAS* mutation was significantly associated with poor survival on multivariate analysis. Other targetable mutations included *PIK3CA* (n = 2) and *ALK* (n = 1). On NGS, 26 mutations were noted in 15 cases. *TP53* and PI3 kinase pathway (*STK11*, *RICTOR*, *TSC2*) mutations were common. One case had *FGF10* amplification, whereas another had *FGF3-TACC* gene fusion, not previously described in gallbladder cancer. In conclusion, somatic mutation profiling using archival FFPE samples from gallbladder cancer is feasible. NGS, in particular, may be a useful platform for identifying novel mutations for targeted therapy.

1. Introduction

Gallbladder cancer affects more than 140,000 patients annually worldwide; and more than 100,000 will die each year from this disease [1]. Women are affected more than men; and in the United States, Hispanic populations and Alaskan natives have a disproportionately high incidence of

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gallbladder cancer [2]. There is a remarkable geographic variation, with the highest incidence rates reported in India, Korea, Japan, Czech Republic, Slovakia, Spain, Columbia, Chile, Peru, Bolivia, and Ecuador. Etiologies include chronic cholelithiasias, *Salmonella* infections, toxin exposure, and obesity; and rarely, it is due to genetic diseases like hereditary nonpolyposis cancer coli and type 1 neurofibromatosis. Gallbladder cancer is thought to be at least partly the consequence of chronic inflammation–induced genetic changes.

The current molecular profiling data of gallbladder cancer are limited to small case series or case reports that include one or more oncogenes. High-throughput screening for targetable mutations in this disease is lacking. An understanding of the molecular characteristics and heterogeneity of gallbladder cancer is critical towards improving the treatment paradigm for this disease. An impetus for such characterization is the potential of targeted therapies directed against the products of these molecular aberrations including the tumor proteomic profile. Once the underlying molecular abnormalities of a cancer are identified, targeted inhibitors can be discovered and result in incremental benefit even in genetically heterogeneous malignancies. For instance, in lung cancer, the identification of echinoderm microtubule associated protein like 4–anaplastic lymphoma kinase (*EML4-ALK)* mutation has led to a targeted approach with crizotinib; and tumors with epidermal growth factor receptor (*EGFR*) mutations, to the development of erlotinib or gefitinib [3]. High-throughput technologies that can rapidly screen for somatic mutations in archival formalin-fixed, paraffin-embedded (FFPE) specimens are critical for this effort. The Sequenom MassArray system is ideally suited for the detection of low-abundance mutations and can be customized towards targeted therapeutics [4,5]. In the present study, we used the high-throughput Sequenom MassArray approach to investigate mutations in 33 genes in a cohort of gallbladder cancer cases to determine the frequency of genetic mutations in this population. We also explored next-generation sequencing (NGS) to examine a wider panel of genetic aberrations in a limited number of gallbladder cancer cases.

2. Materials and methods

2.1. Tumor samples

Surgically resected, FFPE specimens were obtained for 72 patients with gallbladder cancer. The paraffin-embedded blocks were sectioned, and hematoxylin and eosin (H&E)–stained slides were reviewed by surgical pathology to confirm the tumor content in each section. Ten serial sections (4 μm) were cut from selected tissue blocks, and areas with tumor tissue were microdissected from those slides using the H&E slides as templates. Approval for the study was obtained from the institutional review board at MD Anderson Cancer Center.

2.2. DNA extraction

The samples were deparaffinized using xylene washes followed by ethanol (100%) washes. DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) according to the manufacturer protocol. DNA was quantitated using the NanoQuant system (Tecan Group, Männedorf, Switzerland).

2.3. Sequenom MassArray

Hot spot mutational analysis was performed using the Sequenom MassArray using the iPLEX technology (Sequenom, San Diego, CA). This technology allows for parallel high-throughput screening while using minimal DNA obtained from FFPE specimens [6]. Mutations were screened by using amplification through polymerase chain reaction (PCR) and single-base primer extension where the wild-type or mutated base was identified by mass spectrometry. Briefly, for each mutation site, PCR and extension primers were designed using Sequenom Assay Design. PCRs were run following the manufacturer's protocol. After PCR, amplicons were cleaned using EXO-SAP kit (Sequenom, San Diego, CA, USA) in a GeneAmp 9700 thermocycler (Applied Biosystems, Grand Island, NY, USA). The primer was then extended by iPLEX chemistry, desalted using Clean Resin (Sequenom), and spotted onto SpectroChip matrix chips (Sequenom) using a nanodispenser (Samsung, San Diego, CA, USA). Chips were run in duplicate on a Sequenom MassArray matrix-assisted laser desorption/ionization time-of-flight MassArray system. We used Sequenom Typer Software for visual inspection and interpretation of mass spectra. Reactions where the mutant peak represented more than 10% of the wild-type peak were scored as positive. The data analysis was performed using MassArray TYPER 4.0 genotyping software (Sequenom) where the single nucleotide polymorphism (SNP) calls were divided into 3 groups—conservative, moderate, and aggressive calls—depending on the level of confidence.

The Sequenom panel used here was previously designed by the Characterized Cell Line Core (Core Shared Resources; CCSG) at MD Anderson Cancer Center with the aim of detecting somatic DNA alterations in cancer samples. The Sequenom panel was designed based on data from the Catalogue of Somatic Mutations in Cancer and The Cancer Genome Atlas that reported those alterations (and others in the panel) as somatic mutations previously. A total of 159 point mutations in 33 genes frequently mutated in solid tumors including were analyzed. The analytical sensitivity of the assay (limit of detection [LOD] 5%-10% of mutant DNA in total DNA) is higher than conventional Sanger sequencing (LOD, 10%-20%) and similar to pyrosequencing (LOD, 5%-10%). The advantages offered by the