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ORIGINAL ARTICLE

Intrahepatic cholangiocarcinoma and gallbladder cancer: distinguishing molecular profiles to guide potential therapy

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Abstract

Background: Chemotherapy regimens for intrahepatic cholangiocarcinoma (ICC) and gallbladder adenocarcinoma (GC) remain interchangeable; however, response rates are frequently suboptimal. Biomarkers from ICC and GC patients were interrogated to identify actionable differences with potential therapeutic implications.

Methods: From 2009 to 2012, pathological specimens from 217 ICC and 28 GC patients referred to Caris Life Sciences were evaluated. Specific testing by immunohistochemical analysis for 17 different biomarkers was performed.

Results: In the collective cohort (n = 245), actionable targets included: 95% low thymidylate synthase (TS), 82% low ribonucleotide reductase subunit M (RMM) 1 and 74% low excision repair cross complementation group (ERCC) 1, indicating potential susceptibility to fluoropyrimidines/capecitabine, gemcitabine and platinum agents, respectively. Additional targets included TOPO1 (53.3% high, Irinotecan), MGMT (50.3% low, temozolomide), TOP2A (33% high, anthracyclines) and PGP (30.1% low, taxanes). Subgroup analysis by tumour origin demonstrated a differential biomarker expression pattern with a higher frequency of ICC tumours showing low levels of TS (99% versus 72%, $P < 0.01$), and RRM1 (85% versus 64%, $P = 0.02$) when compared with GC. Conversely a greater frequency of GC demonstrated high levels of TOPO1 (76% versus 50%, $P = 0.02$) versus ICC, indicating a potential increased benefit from irinotecan.

Discussion: Differences in the molecular profiles between ICC and GC provide evidence that the two are distinct diseases, requiring different treatment strategies to optimize a response.

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Introduction

Intrahepatic cholangiocarcinoma (ICC) and gallbladder adenocarcinoma (GC) are frequently considered a similar disease in treatment planning. ICC is the second most common primary malignant liver tumour and incidence rates have been increasing in the United States and worldwide.[1,2] Gallbladder adenocarcinoma, while rare among western countries, is the most common malignancy of the biliary tract and shows a geographical variance, occurring more frequently in northern India, Japan and Chile.[3,4] For both ICC and GC, an R0 surgical resection is the only potentially curative treatment; however, both diseases tend to be asymptomatic in the early stages and few patients present early enough to be considered surgical candidates.[5,6]

For many patients diagnosed with ICC and GC, chemotherapy is the only treatment option. According to the National Comprehensive Cancer Network (NCCN), first-line regimens for both ICC and GC are interchangeable, despite the two being recognized as separate diseases. Accepted regimens include fluoropyrimidine-based, gemcitabine-based, or gemcitabine/cisplatin

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combination therapy for advanced or unresectable disease.[7] However, suboptimal response rates as evidenced by a median survival of less than a year, underscore the need for more effective treatment regimens.[8,9]

Research into the molecular pathogenesis of both ICC and GC has revealed potential mechanisms contributing to tumourigenesis. Epidermal growth factor receptor activation in the setting of chronic inflammation, KRAS and IDH1 mutations, as well as epigenetic and chromosomal abnormalities have all been implicated in the development of ICC.[10] While GC has not been as thoroughly studied, mutations in KRAS, p53, increased COX2, microsatellite instability and decreased adhesion molecules have all been proposed to contribute towards tumourigenesis.[11] Recently, whole exome sequencing of GC showed mutations in the ErbB pathway in 36% of tumours analysed, and found the mutations correlated with a poor prognosis.[12] Despite these advances, much is still unknown about the molecular profiles.

Many chemotherapeutic agents, however, have been extensively studied across multiple tumour types, yielding insight into their mechanisms of action, as well as the mechanisms of susceptibility and resistance. Clinical susceptibility to fluoropyrimidines is associated with a low expression of thymidylate synthase (TS),[13] susceptibility to gemcitabine is associated with low expression levels of ribonucleotide reductase subunit M1 (RRM1) [14] and susceptibility to platinum agents, such as cisplatin, are associated with low expression of excision repair cross complementation group 1 (ERCC1).[15] The use of all three of these drugs is recommended in advanced ICC and GC. Thus, information about the expression of TS, RRM1 and ERCC1 has a potential theranostic value.

Biomarker analysis of actionable targets known to convey susceptibility to specific drugs has been purported to be an effective method of tailoring existing chemotherapeutic agents to exploit the specific weaknesses in individual tumours.[16,17] Studies have demonstrated that molecular profile-guided therapies can provide improved response rates across multiple tumour types.[18] This study sought to differentiate the molecular profiles of ICC and GC by a panel of biomarkers to evaluate the potential efficacy of current chemotherapy regimens and potentially refine current treatment strategies.

Patients and methods

From 2009 to 2012, pathological specimens from 217 ICC and 28 GC patients were referred to Caris Life Sciences, a commercial referral diagnostic laboratory, for molecular profiling aimed at providing theranostic information. The diagnoses and tissue samples were collected from referring physicians according to pathology and clinical history. This de-identified data were obtained directly from Caris Life Sciences. As the data was de-identified, patient consent was not required.

Immunohistochemistry

Specific testing by Immunohistochemistry (IHC) was performed for 17 different biomarkers using the following antibodies: AR (AR441/AR318), BCRP (6D171), cKIT (polyclonal), ERCC1 (8F1), ER (SP1), Her2 (4B5), MGMT (MT23.3), MRP1 (33A6), PGP (C494), PR (1E2/100), PTEN (6H2.1), RRM1 (polyclonal), SPARC monoclonal (122511), SPARC (polyclonal), TOPO1 (1D6), TOPO2A (3F6) and TS (TS106/4H4B1). IHC analysis was performed on formalin-fixed paraffin-embedded tumour samples using commercially available detection kits, automated staining techniques (Benchmark XT; Ventana, Tucson, AZ, USA; and AutostainerLink 48; Dako, Carpinteria, CA, USA) in a CLIA/CAP certified, ISO validated lab (Caris Life Sciences, Phoenix, AZ, USA). Staining intensity was scored 0, 1+, 2+ or 3+, and the percentage of stained cells (0–100%) was assessed by board-certified pathologists. Results were then categorized into positive or negative by defined thresholds specific to each marker based on published evidence (Supporting information).

Institutional Review Board

We obtained Institutional Review Board approval to retrospectively review and analyse the data collected from the pathological specimens described above.

Statistical analysis

Categorical variables were described as totals and frequencies. Comparison between subgroups was analysed using a two-sided, Fisher's exact test for categorical data and a two-sided Mann–Whitney *U*-test for continuous variables. Alpha was set at 0.05.

Results

In total, 245 tissue samples were analysed; 217 IHC and 28 GC. The median age of the total cohort was 58 years, with a slight female preponderance (n = 133, 54%). By subgroup, the median age for ICC patients was 58 years, and 59 years for GC patients (P = 0.373). Both subgroups showed a female preponderance, however, it was much more pronounced in the GC subgroup (n = 20, 71%) as compared with the ICC subgroup (n = 113, 52%; P = 0.069).

Biomarker analysis of actionable targets

IHC analysis of biomarkers associated with first-line chemotherapy agents among the total cohort found TS expression to be low in 96% (fluoropyrimidines), low RRM1 expression in 82% (gemcitabine) and low ERCC1 expression in 74% (Cisplatin, Table 1). Additional non-NCCN compendium agents and their associated biomarkers were also analysed. Among the total cohort, potential susceptibility to irinotecan, temozolomide, nab-paclitaxel and epirubicin occurred at lower