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ORIGINAL ARTICLE

Concomitant dysregulation of microRNAs miR-151-3p and miR-126 correlates with improved survival in resected cholangiocarcinoma

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Abstract

Background: MicroRNAs (miRNAs) are small non-coding genes which become dysregulated in cancer and may predict survival. The role of miRNAs in outcomes in cholangiocarcinoma (CC) has not been reported.

Methods: RNA was extracted from 32 resected CCs along with adjacent uninvolved bile duct epithelium. A total of 43 miRNAs were quantified using NanoString™. Clinicopathologic characteristics and outcomes were captured and compared. Overall survival curves were created using the Kaplan–Meier method; factors, including miRNA expression, were compared by log-rank, chi-squared or Cox regression analyses.

Results: Absolute expression of each miRNA was compared with overall survival after excluding perioperative deaths ($n = 3$). One upregulated (miR-151-3p; $P = 0.003$) and one downregulated (miR-126; $P = 0.023$) miRNA in resected CC relative to adjacent normal bile duct epithelium correlated with survival on univariate analysis. Clinical factors and these miRNAs were compared. Dysregulated miR-151-3p and miR-126, respectively, were the only factors that correlated with improved overall survival [41.5 months vs. 12.3 months ($P = 0.002$) and 21.9 months vs. 15.1 months ($P = 0.02$), respectively]. In eight patients, both miRNAs were dysregulated. In the remainder, only one or neither showed dysregulation. Concomitant dysregulation correlated with the best overall survival (58.7 months vs. 15.1 months; $P < 0.000$; $n = 8$); clinicopathologic factors in these groups were otherwise similar.

Conclusions: In resected CC, the concomitant dysregulation of both miR-151-3p and miR-126 was the factor related to the greatest improvement in overall survival. Further analysis of the targets of these miRNAs may yield potential therapeutic targets or prognostic biomarkers.

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Introduction

Cholangiocarcinoma (CC) is a primary hepatic malignancy originating from bile duct epithelium. It is the second most common primary hepatic neoplasia and, although rare, accounts for approximately 3% of all gastrointestinal malignancies.[1] Over 3500 cases will be diagnosed in the USA this year alone. Further, the worldwide incidence of CC and associated mortality rates are increasing.[2] Hepatobiliary cancer accounts for 13% of the 7.6 million cancer-related deaths worldwide each year; of these, CC accounts for 10–20%.[3] Because of its poor prognosis, incidence and prevalence are essentially the same.

Risk factors for CC include chronic inflammation, primary sclerosing cholangitis (PSC), infestation with liver flukes, congenital disorders (e.g. choledochal cysts), hepatolithiasis, viral hepatitis, and lifestyle choices (i.e. ethanol use, obesity, smoking). Most patients with CC have no identifiable risk factors. Effective screening tools for CC are lacking and the disease remains difficult to diagnose. In patients identified as high risk (i.e. patients with

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chronic viral hepatitis and PSC), there are no reliable screening methods. Even histopathology can be deficient in making a diagnosis after resection or biopsy.

The only definitive curative therapy is surgical resection in those patients without distant or locally advanced disease. Despite aggressive resection, overall survival is poor and 5-year survival rates range from 20% to 35%.[4–9] In patients with advanced disease, survival remains limited.[10] In patients with advanced disease, the standard of care includes gemcitabine-based chemotherapy or enrolment in a clinical trial. Growing understanding of the molecular and cellular aetiology of this disease is enabling the development of novel targeted therapies. However, little is known about the pathogenesis of CC.

MicroRNAs (miRNAs) are small (20–22 nucleotides), non-coding RNA fragments that have critical functions in various biological processes. To date, over 1000 miRNAs have been reported and shown to play a role in cell proliferation, apoptosis and differentiation. They are linked to oncogenesis through their function as oncogenes or tumour suppressors.[11]

The present authors have noted anecdotally that a subset of patients undergoing resection for CC appear to do better than others for unclear reasons. Therefore, this group hypothesized that key miRNAs can be utilized to characterize a subset of patients with resectable CC in whom overall survival will be improved and that these miRNAs will thus be prognostic. Further, these miRNAs modulate protein expression involved in tumour growth and may serve as potential targets for future therapies.

Materials and methods

Institutional review board approval was obtained for the retrieval of data from the archival files of the Department of Pathology at this institution. A total of 135 patients presenting with CC between 1993 and 2007 were subsequently identified. Of these, 90 underwent surgical exploration. Of the 69 who underwent resection with curative intent, tissue samples for 32 patients were of adequate volume for RNA extraction. Three 2-mm cores of tumour and matched adjacent benign bile duct epithelium were punched from paraffin blocks. Deparaffinization using 100% xylene at 50 °C for 3 min was followed by RNA extraction using the RecoverAll™ Total Nucleic Acid Isolation Kit (Ambion®, Life Technologies, Corp., Grand Island, NY, USA) according to the manufacturer's guidelines. Calculation of the miRNA using the NanoString nCounter® Analysis System (NanoString Technologies, Inc., Seattle, WA, USA) was performed according to the manufacturer's protocol. This method is described elsewhere.[12] Selected miRNAs were validated by reverse transcription polymerase chain reaction (RT-PCR) using standard protocols and are reported elsewhere.[13]

Statistical analysis

NanoString nCounter® provides a quantitative assessment of miRNA expression. Dysregulated miRNA expression was defined as aberrant expression compared with that in normal adjacent bile duct epithelium. The expression of each dysregulated miRNA was dichotomized as 'high' or 'low' relative to the mean tumour expression of each miRNA. These data were then correlated with clinical outcomes.

The Kaplan–Meier method was used to construct overall and progression-free survival curves. Time-to-event variables were calculated from the time of resection. Log-rank analysis was used to compare groups. Perioperative deaths were considered as deaths that occurred within 60 days of resection or during the index admission and were excluded from all survival and recurrence analyses. Progression was determined by the date when either intra- or extrahepatic progression of disease was noted radiographically. Categorical variables were analysed using Pearson's chi-square or Fisher's exact test as appropriate. Student's *t*-test was used to analyse continuous variables. All tests were examined at a two-tailed significance level of 0.05. Cox regression multivariate analysis was used to compare factors associated with survival. All factors were included and analysed in a forward stepwise fashion. Logistic regression multivariate analysis was used to compare associations with the differential expression of miRNAs of interest. All factors were included and analysed in a forward stepwise fashion; however, because data for carbohydrate antigen 19-9 (CA 19-9) were missing for 12 patients, this factor was excluded from the final analysis.

Table 1 Demographic data for patients undergoing resection for cholangiocarcinoma (n = 32)

Characteristics	Data
Age, years, median (range)	66 (47–85)
Gender, male, *n*	12
Tumour location, *n*	Intrahepatic, 11
	Hilar, 8
	Distal, 13
Elevated CA 19-9, *n*	15
CA 19-9, mL, median (range)	51.3 (<15–9000)
Elevated bilirubin, *n*	13
Adjuvant chemotherapy, *n*	10/29

CA 19-9, carbohydrate antigen 19-9.

Results

A total of 32 patients with CC resected with curative intent for whom adequate pathologic specimens were available for miRNA analysis were identified. Patients were, on average, in their seventh decade (Table 1). The majority were female and had extrahepatic tumours, eight of which were hilar. Almost half of the patients had elevated CA 19-9 and/or obstructive jaundice preoperatively (Table 1).

The majority of the patients (n = 20, 63%) were classified as having stage I or II disease (American Joint Commission on Cancer, Cancer Staging Manual, 7th edn).[14] Thus, only about one