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Case report

Patent-Derived Xenograft (PDX) Studies of Intrahepatic Cholangiocarcinoma Indicate Potential Benefit with Trametinib

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Summary

The current study was aimed to analyze the tumor tissue of cholangiocarcinoma from patient to identify the biomarkers at the protein level and mRNA level in cancer tissue. The tumor sample was analyzed by gene expression microarray analysis, DNA sequencing and immunohistochemistry. Gene expression analysis showed 935 genes differentially expressed in cancer tissue compared to adjacent normal. Among them SSP1, TPD52, RAB25, vitronectin, TM4SF4, PDGFRA, PDGFRB are some of the genes with relevance to tumor development and progression. Based on biomarkers expressed in cancer tissue we tested the patient's tumor-derived cancer cells with drugs of potential benefit *in vitro and in vivo*. The *in vivo* data was based on patient derived xenograft (PDX) studies in mice. Tyrosine kinase inhibitor trametinib significantly killed tumor cells *in vitro* and regressed tumor growth *in vivo* compared to other tyrosine kinase inhibitors. Our observation that trametinib regresses cholangiocarcinoma tumor in mice was probably by inhibiting rab25 a (ras oncogene family member) signaling pathway. However, this needs further studies to understand if this hypothesis is true.

Abstract

Background

Cholangiocarcinoma (CCA) is a deadly cancer of the bile ducts with poor prognosis. The incidence rate has been on the rise. In spite of advancement in cancer treatments the survival for CCA patients has little impact. Molecular studies to identify the target proteins to treat cancers have been effective in the recent past. Therefore, analyzing CCA tumors at the gene expression level may lead to the identification of unique proteins that can be targeted for a better treatment. We tested the tumor sample of intrahepatic cholangiocarcinoma patient for gene expression and correlate it to chemosensitivity *in vitro* and *in vivo*.

Methods

Tumor sample analyzed for gene expression by microarray analysis. Tumor cells were cultured and tested for sensitivity against various chemo and targeted drugs. Fresh tumor tissue was used for patient derived xenograft (PDX) studies in NOD scid mice.

Results

Gene expression studies using microarray analysis showed 935 genes to be differentially expressed in tumor tissue compared to adjacent normal. Several genes implicated in various metabolic pathways were altered including those related to cell proliferation and growth. SSP1, CKS2, TM4SF4, TPD52, vitronectin, rab25, VDR, PDGFRA, PDGFRB, PTGS2 were some of the cancer related genes overexpressed while BRCA2 was under expressed. PDX studies for tumor sensitivity test indicated that gemcitabine and trametinib were most effective drugs in tumor regression.

Conclusion

We found that gemcitabine, premetrexate, paclitaxel and cisplatin were effective in killing cancer cells *in vitro*. Among targeted therapy drugs tested, trametinib was most effective *in vitro* and *in vivo* (PDX) studies. Trametinib though it is approved for treating metastatic melanomas seems to be a promising drug to treat CCA.

Keywords: Cholangiocarcinoma; Chemotherapy; PDX; Trametinib; molecular analysis; targeted therapy

Introduction

Cholangiocarcinoma (CCA) is the most common biliary malignancy and the second most common hepatic malignancy following hepatocellular carcinoma [1] and the incidence rate has been increasing over the past three decades [2]. CCA is a slow-growing tumor that metastasizes late during cancer progression and presents with symptoms of cholangitis due to blockage of the bile duct by tumor growth [3]. In the United States, Hispanics and Asians have the highest incidence rate of CCA, whereas African Americans have the lowest [4].

The etiological causes of CCA have been attributed to primary

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sclerosing cholangitis (PSC) [5], liver fluke infection [6], pancreaticobiliary maljunction [7] and chemical exposure [8]. There are several mutations in oncogenes and tumor suppressor genes such as tumor protein 53, KRAS and SMAD family member 4 that have been reported [9]. Standard chemotherapy has had little impact on increasing the overall survival of cholangiocarcinoma patients in spite of several advances in the field [10,11]. Response rates with 5-FU have been 10% at best with a median survival of 6 months. Several new combination regimens were tried in advanced cholangiocarcinoma [12]. Gemcitabine and docetaxel gave a response rate of only 9% with advanced cholangiocarcinoma or gall bladder cancer with median survival of 11 months [13]. Other combinations like 5-FU/cisplatin [14], gemcitabine/cisplatin [15], gemcitabine/capecitabine [16], gemcitabine/docetaxel [13], have only improved survival by a few months.

Apart from chemotherapeutic drugs, there has been a focus on developing targeted therapies against cancers especially tyrosine kinase inhibitors. Many tumor cells harbor point mutations within certain genes that constitutively upregulate kinase activity [17]. Application of selective kinase inhibitors based on genomic information in clinical oncology has shown great promise in improving patient outcome. In spite of a tremendous clinical benefit of some agents, patients who initially respond to targeted therapeutics commonly relapse. Therefore, there is a need for identifying new drug targets to attack the cancers. The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) have identified several new mutations in kinases in cancer [18,19]. For example a hotspot mutation that upregulates the kinase activity found prevalently across oncogenic kinases is BRAF V600 [20]. Mitogen-activated extracellular signal-regulated kinase (MEK) activity is critical for mutant BRAF signaling. In preclinical models of human melanoma, selective MEK inhibitors have inhibited growth and induced cell death in tumors bearing either BRAF or NRAS mutations [21]. Trametinib is a reversible and highly selective allosteric inhibitor of MEK1 and MEK2 with anticancer activity against BRAF V600 mutation carrying metastatic melanoma. In xenografts tumor models, trametinib showed sustained growth inhibition in tumor lines [22]. Therefore, we tested this drug in our studies with patient derived tumor xenograft along with standard chemotherapeutic drugs like gemcitabine.

Materials and Methods

The cell culture medium consisted of Dulbecco's Modified Eagles Media (DMEM) with 4.5 g/L glucose and L-glutamine (Lonza, NJ), 10% (v/v) fetal bovine serum and penicillin/streptomycin (10 U Pen/mL, 10 µg Strep./mL). All cytotoxic agents were obtained from Selleckchem, TX, USA and dissolved in DMSO to prepare 10mM stock solutions. Collagenase was purchased from Sigma-Aldrich (cat#C5138). Stepwise dilution of the stock in the cell culture medium yielded solutions at test concentrations. Cholangiocarcinoma cells were derived from patient's fresh tumor sample. For *in vivo* studies, fresh tumor sample was stabilized by implanting the tumor tissue in kidney capsule of NOD scid mice. After stabilization, the tumor from the kidney capsule was obtained and minced to implant subcutaneously in different groups of NOD scid mice. After the tumor showed stabilized growth subcutaneously the mice were treated with drugs. Cell Counting Kit-8 (Dojindo, Japan) was diluted in DMEM (2.5%) to yield the viable cell counting solution. A Molecular Device UV-Max microplate reader was used to collect absorbance data at 450 nm. An approval was obtained from the patient's family to perform studies and publish the data.

Establishment of Primary Cells

Fresh tumor was minced using sterile scalpel blade and digested with 0.5% collagenase and incubated at 37°C in water bath with shaking from 30min to 1hr. digested tissue was filtered into a fresh tube containing DMEM medium. Cells were centrifuged at 1500 RPM for 5min. Cell pellet was resuspended in 10ml DMEM medium with 10% FBS and 1% pen/strep. Cells were counted and seeded in cell culture plate previously coated with 2% gelatin and incubated in CO_2 incubator.

Cell Proliferation Assay and Cytotoxicity Assay

Patient-derived primary tumor cells were seeded (~10,000 cells/well) in a 96-well plate in the cell culture medium and incubated in a humidified CO_2 chamber for 24 hours. 100 µl of cytotoxic agents at various concentrations were added in each well. The plates were then incubated for 48 hours. The medium was discarded and 100 µL of cell counting solution was added to each well. After 2 hour incubation, absorbance was measured at 450 nm in the microplate reader. The data was plotted in GraphPad Prism software.

Biomarker Analysis

The biomarker analysis performed at Caris Life Sciences, Phoenix, AZ was by either immunohistochemistry, next generation sequencing (NGS) or by microarray analysis on formalin fixed paraffin embedded (FFPE) tissue sections. Gene expression studies by microarray analysis on fresh frozen tumor and adjacent normal tissue on Affymetrix Human Gene 1.0 ST arrays was performed by Genome Explorations, Memphis, TN. Fresh tissue was used to prepare total RNA and reverse transcribed to cDNA that was used on microarray chips.

Patient derived xenograft study

Patient derived tumor xenograft studies in mice were performed at Champions Oncology, Baltimore, MD. by following the institutional animal care and use committee approved protocols. Fresh tumor tissue of CCA was transplanted (fragments of 1.5mmx1.5mm in size) under the renal capsule of NOD scid mice. After 6-8 weeks, tumors were excised and serially transplanted into additional mice subcutaneously for drug testing. The study protocol and drug doses were used with some minor modifications of previous studies [23, 25]

The tumor was allowed to grow stably before testing the drugs. Beginning Day 0, tumor dimensions were measured. Later, tumor dimensions were measured twice weekly by digital caliper and data, including individual and mean estimated tumor volumes (Mean TV ± SEM), are recorded for each group. Tumor volume was calculated using the formula: $TV = width^2 \times length \times \pi/2$. At completion of the

study on day 22, percent tumor growth inhibition (%TGI) values were calculated and reported for each treatment group (T) versus control (C) using initial (i) and final (f) tumor measurements by the formula: $\%TGI=1-(T_f-T_i)/(C_f-C_i)x100$. Individual mice reporting a tumor volume >120% of the Day 0 measurement are considered to have progressive disease (PD). Individual mice with neither sufficient shrinkage nor sufficient tumor volume increases are considered to have stable disease (SD). Individual mice reporting a tumor volume ≤70% of the Day 0 measurement for two consecutive measurements over a seven day period are considered partial responders (PR). If the PR persisted until study completion, percent tumor regression (%TR) is determined using the formula: $\%TR= (1-T_f /T_i)x100$; a mean value is calculated for the entire treatment group. Individual mice lacking palpable tumors for two consecutive measurements over a seven day period are classified as complete responders (CR). The clinical specificity for this test is 60%; the clinical sensitivity of this test is 98.1%.

Statistical Analysis

Statistical analysis was carried out using the two-tailed, unpaired Student's t test. A p-value less than 0.05 was considered statistically significant. Data are expressed as mean±S.D. For microarray analysis by Genome Explorations, background correction, normalization, and summarization was done by the RMA method. Signal values were Log2 transformed. Later filtered for probe sets with Log2 fold change values < -1 or > +1 (2-fold change) for any pair wise comparison. The lack of replicate samples limited any estimates of statistical significance.

Results

In Vitro Drug Sensitivity of Tumor Derived Primary Cells

Cultured primary cells from patient's tumor were treated with different chemotherapeutic drugs to identify the relative sensitivity of tumor cells using CCK8 assay kit. The results indicated that the cancer cells were most sensitive to paclitaxel followed by premetrexate, gemcitabine, cisplatin, irinotecan, Ara-C, carboplatin, 5-FU and oxaliplatin in order of decreasing effectiveness. Temozolomide, cyclophosphamide and dexamethasone were ineffective in killing CCA cells. Trametinib showed best cytotoxic activity among the tyrosine kinases tested. Sorafenib and sunitinib showed moderate efficacy against tumor cells while gefitinib, imatinib, pazopanib and erlotinib were ineffective in *in vitro* (Table 1).

Biomarker Analysis

The tissue samples were analyzed for detecting biomarkers across hundreds of hotspots in cancer genomes. The results from Caris Profiling indicated over expression of biomarkers (Table 2). The over expression of these biomarkers in cancer tissue implicated a clinical benefit from using chemo agents like cisplatin, carboplatin, oxaliplatin, gemcitabine, sunitinib, sorafenib and celecoxib. The results of the microarray data indicated 935 genes altered in tumor tissue compared to adjacent normal. Most prominently up regulated genes involved in cell division and proliferation are listed in Table 2. We identified certain novel genes up regulated in cancer tissue implicated in cell proliferation and growth such as tumor protein

Table 1: Drug sensitivity tested on patient-derived tumor cells

DRUGS	EC_{50} mM
Paclitaxel	0.0001 ± 0.00001
Premetrexate	0.0026 ± 0.0003
Cisplatin	0.31 ± 0.035
Irinotecan	0.42 ± 0.071
Ara-C	0.98 ± 0.17
Carboplatin	1.37 ± 0.12
5-FU	1.68 ± 0.71
Oxaliplatin	2.09 ± 0.21
Sorafenib	2.20 ± 0.36
Sunitinib	2.76 ± 0.39
Gefitinib	7.39 ± 1.55
Temozolomide	92.7 ± 78.5
Erlotinib	88.3 ± 79.7
Cyclophosphamide	112 ± 30
Dexamethasone	226 ± 77
Gemcitabine	0.096 ± 0.026
Imatinib	15.2 ± 0.6
Pazopanib	102 ± 40
Trametinib	0.066 ± 0.048
Berberine	7.2 ± 2.4
Gossypol	5.5 ± 1.9
Parthenolide	1.5 ± 0.7

Table 2: Biomarker analysis from patient tumor sample

Biomarker	Result	Analyzed by
ERCC1	Over expressed	Caris Life Sciences
MGMT	Over expressed	Caris Life Sciences
RRM2	Over expressed	Caris Life Sciences
VDR	Over expressed	Caris Life Sciences
PDGFRB	Over expressed	Caris Life Sciences
BRCA2	Under expressed	Caris Life Sciences
PTGS2	Over expressed	Caris Life Sciences
SSP1	Over expressed	Genome Explorations
CKS2	Over expressed	Genome Explorations
Cyclin b1	Over expressed	Genome Explorations
PTTG1	Over expressed	Genome Explorations
PCNA	Over expressed	Genome Explorations
PDGFRa	Over expressed	Genome Explorations
Vitronectin	Over expressed	Genome Explorations
TM4SF4	Over expressed	Genome Explorations
TPD52	Over expressed	Genome Explorations
RAB25	Over expressed	Genome Explorations

52 (TPD52), transmembrane 4 L six family member 4 (TM4SF4), vitronectin and Rab25 (a RAS oncogene family member). Among them, platelet derived growth factor receptor (PDGFRa) was one of the important up-regulated genes that could be targeted by sorafenib, sunitinib or imatinib. We tested the patient-derived tumor cells with marker-associated beneficial drugs for sensitivity.

Patient derived xenograft studies

To have a better understanding of the drug sensitivity of the tumor, freshly resected tumor was used in PDX studies in mice. The mice were divided into different groups with each group consisting at least three mice. Tumor bearing mice were treated with imatinib, gemcitabine, pazopanib and trametinib that were predicted to have clinical benefit based on the results of previous molecular analysis. The number of animals in each group, drug dosage and the route of drug administration are given in Table 3. Pazopanib targets PDGFR and is approved for advanced soft tissue sarcoma and renal cell carcinoma whereas trametinib, a MEK inhibitor is approved for melanoma but is being investigated in bile duct cancers (www.cancer.org/cancer/bileductcancer/detailedguide/bile-duct-cancer-new-research). The results indicated a relatively better anti-tumor activity with gemcitabine and trametinib as single agents (Figure 1A). The PDX study results for drug efficacy correlate well with *in vitro* drug sensitivity results (Figure 1B and 1C). Tumor regression (TR) of 40% was achieved following treatment with gemcitabine, with tumor growth inhibition (TGI) of 156%, two animals showing partial response (PR) and one animal showing stable disease (SD). Treatment with Trametinib resulted in TR of 18%, TGI of 127%, one animal showing PR and four animals showing SD. Pazopanib and imatinib did not show anti-tumor response. As single agents

Table 3: PDX experimental design

Group	-n-	Agent	Dose (mg/kg/dose)	ROA/Schedule
1	9	Control		
2	3	Gemcitabine	100	i.p./q7dx3
3	3	Imatinib	40	p.o./qdx21
4	5	Trametinib	1	p.o./qdx21
5	5	Pazopanib	40	p.o./qdx21
6	3	Gossypol	50	p.o./qdx21
7	3	Parthenolide	5	i.p./2wklyx3
8	3	Berberine	10	p.o./qdx21

ROA – Route of Administration

i.p. – intra peritoneal

p.o. – by mouth (oral)

qdx21 – one dose every day for 21 days

q7dx 3 – one dose every seven days times 3

2wklyx3 – two doses per week times 3 8OA/Scheduleived growth factor receptor (25,dney capsule was obtained ag the CCA cells in the treatment of CCA.be targeted for a

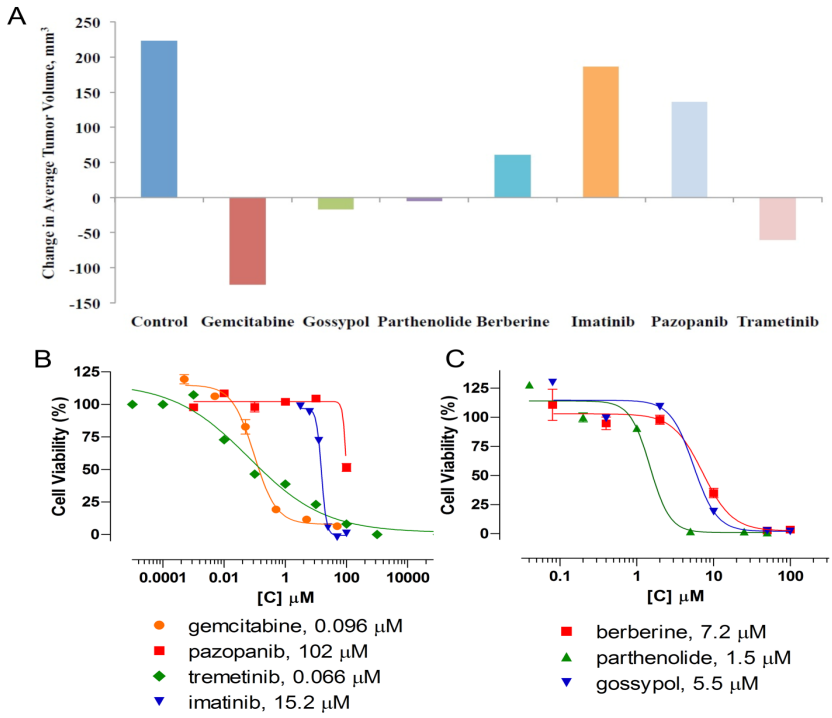

Figure 1: Patient derived tumor xenograft testing for chemosensitivity. NOD scid mice implanted with patient derived tumors were treated with different drugs represented in the figure. Change in tumor volume is represented as bar graph of each drug treatments. Negative y-axis represents tumor regression compared to control (A). Cell viability assay CCK8 data representing the sensitivity of patient derived cancer cells to anti-cancer drug *in vitro* (B &C).

Pazopanib or Imatinib resulted in TGI of 39% and 17%, respectively, with most of the animals showing progressive disease (PD). The results are summarized in Table 4.

Further, we also treated the mice with natural compounds like gossypol, parthenolide and berberine. These drugs were previously shown to have anti-cancer properties [26-28]. Results indicated

Table 4: Patient derived tumor xenograft results summary

Group	% Tumor growth inhibition	RECIST PD/SD/PR/CR*	% Tumor regression
Control		7/2/0/0	n/a
Gemcitabine	156	0/1/2/0	40
Imatinib	17	3/0/0/0	n/a
Trametinib	127	0/4/1/0	18
Pazopanib	39	4/1/0/0	n/a
Gossypol	107	0/3/0/0	n/a
Parthenolide	102	0/3/0/0	n/a
Berberine	73	1/2/0/0	n/a

*PD-Progressive Disease; SD-Stable Disease; PR-Partial Response; CR-Complete Response.

that berberine, gossypol and parthenolide could stabilize the tumor growth to a large extent. As single agents gossypol or parthenolide resulted in TGI of 107% and 102%, respectively. Treatment with berberine resulted in TGI of 73%, with two of three animals showing SD.

Discussion

Cholangiocarcinoma is a type of highly malignant cancer of the biliary tract with poor prognosis. It can be a challenge to diagnose CCA because of its anatomic location and silent clinical character. The clinical manifestations of intrahepatic cholangiocarcinoma include non-specific symptoms like abdominal pain, malaise, fatigue, cachexia and night sweats [29].

Personalization of cancer therapy has been the focus in the field to overcome the inter-individual and tumor variations. Towards personalization of cancer therapy, molecular pathologic investigations are being performed routinely on tumors and targeted drugs are being developed [30]. The aim of chemosensitivity assay is to predict the *in vivo* response and resistance to chemotherapeutics. Earlier studies applied various methods to determine *in vitro* chemosensitivity [31-33] that have indicated a predictive accuracy for *in vivo* sensitivity of 30-86% and resistance of 92% [34,35]. The standard practice of care for an advance staged intrahepatic cholangiocarcinoma is systemic chemotherapy with gemcitabine and cisplatin [36]. In advanced cholangiocarcinoma patients in addition to best supportive care, 5-FU with leucovorin and etoposide based systemic chemotherapy improved median survival by 3.5 months compared to best supportive care alone [37]. Later, more clinical trials with 5-FU based combinations were performed by several groups with an overall survival of 13.3 months being best when treated with leucovorin, 5-FU and cisplatin combination [38]. Gemcitabine based single agent or combination studies showed a median overall survival in the range of 5-14 months [39-41]. Such trials have not yielded superior achievement of improving overall survival beyond a few months.

Development of new techniques enabled the screening of cancer tissue against a range of anti-cancer drugs *in vitro* prior to the start of chemotherapy to patient. Further, immune-compromised transgenic mice enabled successful patient derived xenografts (PDX) studies that work as a great tool to study the tumor sensitivity to drugs *in vivo*. This tool is being used in personalizing the treatment for patients. *In vitro* and *in vivo* models of drug testing will help choose the effective drugs to benefit the patients. Our experiments with PDX studies indicated potential benefit when treated with gemcitabine that served as a positive control for our studies as it is widely used clinically to treat CCA. Our results with imatinib were not encouraging despite its target PDGFRA was over expressed in tumor cells making it difficult to explain why imatinib treatment had poor response. However, the other tyrosine kinase inhibitor trametinib showed promising results as compared to either imatinib or ponatinib. Our study with natural compounds like gossypol, parthenolide and berberine indicate benefit in treating tumor bearing mice as the disease was stabilized. Especially the results with gossypol and parthenolide were highly promising indicating their potential as therapeutic drugs for cholangiocarcinoma. PDX results with berberine indicated that it could control the tumor growth to a large extent. Berberine was previously reported to kill cholangiocarcinoma cells specifically [42].

In the current study, based on *in vitro* tumor sensitivity results, paclitaxel and premetrexate were best in killing tumor derived primary cells. Paclitaxel represents the taxane family of drugs that interferes with the normal breakdown of microtubules during cell division. This has been used to treat a number of cancers including ovarian, lung, bladder, prostate, melanoma esophageal, breast as well as Kaposi's sarcoma [43]. Pemetrexed is similar to folic acid and is referred as folate antimetabolite. It inhibits the enzymes involved in DNA and RNA synthesis. Clinically pemetrexed is approved for pleural mesothelioma [44]. It has also been approved for the treatment of non-small cell lung cancer (NSCLC) [45,46]. However, paclitaxel and premetrexate have not been used in the treatment of cholangiocarcinoma. Therefore, these drugs can be further investigated to test whether they can be used in the treatment of CCA.

Gene expression microarray studies are very useful in providing critical information on the molecular biology of the tumor. Many proteins that are important in cell division and tumor growth can be analyzed to identify marker proteins or receptor tyrosine kinases that can be targeted for treatment. Previous studies have reported stromal over expression of osteopontin/SPP1 in cholangiocarcinoma [47]. Another study has demonstrated that the stromal over expression of osteopontin/SPP1 as an independent prognostic marker for overall and disease free survival [48]. The cancer tissue of the present patient in this report had very high expression of SSP1 gene indicating a poor prognosis for overall survival. Further, CKS2 over expression was reported to have poor

prognosis [49]. Knockdown of CKS2 using siRNA was shown to down regulation of cyclin A and cyclin B1 which led to the arrest of cell cycle in G2/M phase and further up regulated Bax and caspase-3 facilitated apoptosis in CCA [49]. Further, down regulation of CKS2 expression sensitized the cells to chemotherapy. PDGFRα is a tyrosine kinase receptor that plays a critical role in cell division and metastasis of tumor. Several groups have shown inhibition of PDGFRα by imatinib [50-52]. In the present case, patient-derived tumor cells showed no response to imatinib treatment either in *in vitro* or *in vivo* studies.

The results with trametinib were very promising in shrinking the tumor. Recent studies indicated that trametinib can target downstream proteins like MEK of ras signaling pathway to control tumor development and progression (http://www.cancer.gov/about-cancer/treatment/research/mek#melanoma).Gene expression in the CCA tumor tissue by microarray analysis found that rab25, which is a member of ras oncogene family, is overexpressed. Therefore, we speculate that trametinib might be targeting rab25 induced signaling pathway in cholangiocarcinoma cells. However, this being a single patient study, the results cannot be generalized. Studies with several samples need to be carried to make specific conclusion. Further, the identification of novel genes over expressed in tumor sample by microarray analysis like TM4SF4, vitronectin and TPD52 may be studied further to understand their use as targets to treat cholangiocarcinoma.

We propose that further studies on trametinib in cholangiocarcinoma cells will provide new insights on the potential use of trametinib as a treatment for cholangiocarcinoma patients. Moreover, choosing appropriate chemotherapy based on the tumor sensitivity tests will help improve the treatment efficacy and overall survival of cholangiocarcinoma patients.

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Authors Contribution

Yao Z, Patel KS, Patel SM, Jadesola A and Elghannam H, performed the research; Andrali SS, Lavinski Y, Sankhala K and Chawla SP, designed the research; Yao Z, Dixit D, Bui E, Hendricks T, Paladiya S, and Everett C, collected and analyzed the data; Yao Z, Chawla SP and Andrali SS wrote the paper.

References

1. Welzel TM, McGlynn KA, Hsing AW, O'Brien TR, Pfeiffer RM (2006) Impact of classification of hilar cholangiocarcinomas (Klatskin tumors) on the incidence of intra- and extrahepatic cholangiocarcinoma in the United States. J Natl Cancer Inst 98: 873–875.
2. Khan SA, Davidson BR, Goldin RD (2012) Guidelines for the diagnosis and treatment of cholangiocarcinoma: an update. Gut 61: 1657–1669.
3. Malhi H, Gores GJ. (2006) Cholangiocarcinoma: Modern advances in understanding a deadly old disease. J Hepatol 45: 856-867.
4. Rizvi S, Gores GJ (2014) Molecular pathogenesis of cholangiocarcinoma Dig Dis 32: 564-569.
5. Razumilava N, Gores GJ, Lindor KD (2011) Cancer surveillance in patients with primary sclerosing cholangitis Hepatology 54: 1842–1852.
6. Sithithaworn P, Yongvanit P, Duenngai K, Kiatsopit N, Pairojkul C (2014) Roles of liver fluke infection as risk factor for cholangiocarcinoma. J Hepatobiliary Pancreat Sci 21: 301–308.
7. Nagai M, Watanabe M, Iwase T, Yamao K, Isaji S (2002) Clinical and genetic analysis of noncancerous and cancerous biliary epithelium in patients with pancreaticobiliary maljunction. World J Surg 26: 91–98.
8. Kubo S, Nakanuma Y, Takemura S, Sakata C, Urata Y,et al. (2014) Case series of 17 patients with cholangiocarcinoma among young adult workers of a printing company in Japan. J Hepatobiliary Pancreat Sci 21: 479-488.
9. Ong CK, Subimerb C, Pairojkul C, Wongkham S, Cutcutache I, et al. (2012) Exome sequencing of liver flukeassociated cholangiocarcinoma. Nat Genet 44: 690–693.
10. Everhart JE, Ruhl CE (2009) Burden of digestive diseases in the United States part III: liver, biliary tract, and pancreas. Gastroenterology 136: 1134–1144.
11. Tyson GL, El-Serag HB (2011) Risk factors for cholangiocarcinoma. Hepatology 54: 173–84.
12. Mazhar D, Stebbing J, Bower M (2006) Chemotherapy for advanced cholangiocarcinoma: what is standard treatment? Future Oncol 2: 509-514.
13. Kuhn R, Hribaschek A, Eichelmann K, Rudolph S, Fahlke J, et al. (2002) Outpatient therapy with gemcitabine and docetaxel for gallbladder, biliary, and cholangiocarcinomas. Invest New Drugs 20: 351–356.
14. Ducreux M, Rougier P, Fandi A, Clavero-Fabri MC, Villing AL, et al. (1998) Effective treatment of advanced biliary tract carcinoma using 5-fluorouracil continuous infusion with cisplatin. Ann Oncol 9: 653–656.
15. Thongparsert S, Napapan S, Charoentum C, Moonprakan S 2005 Phase II study of gemcitabine and cisplatin as first line chemotherapy in inoperable biliary tract carcinoma. Ann Oncol 16: 279–281.
16. Knox JJ, Hedley D, Oza A, Feld R, Siu LL, et al. (2005) Combining gemcitabine and capecitabine in patients with advanced biliary cancer: a Phase II trial. J Clin Oncol 23: 2332–2338.
17. Torkamani A, Verkhivker G, Schork NJ (2009) Cancer driver mutations in protein kinase genes. Cancer Lett 281: 117–127.
18. Lawrence MS, Stojanov P, Polak P, Kryukov GV, Cibulskis K, et al. (2013) Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 499: 214–218.
19. Lengauer C, Kinzler KW, Vogelstein B (1998) Genetic instabilities in human cancers. Nature 396: 643–649.
20. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, et al. (2002) Mutations of the BRAF gene in human cancer. Nature 417: 949–954.
21. Solit DB, Garraway LA, Pratilas CA, Sawai Am, Getz G, et al. (2006) BRAF mutation predicts sensitivity to MEK inhibition. Nature 439: 358–362.

22. Yamaguchi T, Kakefuda R, Tajima N, Sowa Y, Sakai T (2011)Antitumor activities of JTP-74057 (GSK1120212), a novel MEK1/2 inhibitor, on colorectal cancer cell lines in vitro and in vivo. Int J Oncol 39: 23–31.

23. Hidalgo M, Bruckheimer E, Rajeshkumar NV, Garrido-Laguna I, De Oliveira E, et al. (2011) A Pilot Clinical Study of Treatment Guided by Personalized Tumorgrafts in Patients with Advanced Cancer. Mol Cancer There 10: 1311-1316.

24. DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, et al. (2011) Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. Nat Med 17: 1514-1520.

25. Morelli MP, Calvo E, Ordonez E, Wick MJ, Viqueira BR, et al. (2012) Prioritizing Phase I Treatment Options Through Preclinical Testing on Personalized Tumorgraft. J Clin Oncol 30: e45-8.

26. Yun BR, Lee MJ, Kim JH, Kim IH, Yu GR, et al. (2010) Enhancement of parthenolide-induced apoptosis by a PKC-alpha inhibition through heme oxygenase-1 blockage in cholangiocarcinoma cells. Exp Mol Med 42: 787-797.

27. He W, Wang B, Zhuang Y, Shao D, Sun K, et al. (2012) Berberine inhibits growth and induces G1 arrest and apoptosis in human cholangiocarcinoma QBC939 cells. J Pharmacol Sci 119: 341-348.

28. Lian J, Ni Z, Dai X, Su C, Smith AR, et al. (2012) Sorafenib sensitizes (-)-gossypol-induced growth suppression in androgen-independent prostate cancer cells via Mcl-1 inhibition and Bak activation. Mol Cancer Ther 11: 416-426.

29. Blechacz B, Komuta M, Roskams T, Gores GJ (2011) Clinical diagnosis and staging of cholangiocarcinoma. Nat Rev Gastroenterol Hepatol 8: 512-522.

30. Oktay MH, Hui P (2012) Molecular pathology as the driving force for personalized oncology. Expert Rev Mol Diagn 12: 811-813.

31. Schoenfelder M. Das onkobiogramm – ein wegweiser fuer die chemotherapie. Zbl Chir 1974 99: 1198-1208.

32. Hamburger AW, Salmon SE (1977) Primary bioassay of human tumor stem cells. Science 197: 461-463.

33. Koechli OR, Avner BP, Sevin BU, Avner B, Perras JP, et al. (1993) Application of the adenosine triphosphate-cell viability assay in human breast cancer chemosensitivity testing: a report on the first results. J Surg Oncol 54: 119-125.

34. Bertelsen CA, Sondak VK, Mann BD, Korn EL, Kern DH. (1984) Chemosensitivity testing of human solid tumors. a review of 1582 assay with 258 clinical correlations. Cancer 53: 1240-1245.

35. Singh B, Li R, Xu L, Poluri A, Patel S, et al. (2002) Prediction of survival in patients with head and neck cancer using the histoculture drug response assay. Head Neck 24: 437-442.

36. Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, et al. (2010) ABC-02 Trial Investigators. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. N Eng J Med 362: 1273-81.

37. Glimelius B, Hoffman K, Sjödén PO, Jacobsson G, Sellström H, et al. (1996) Chemotherapy improves survival and quality of life in advanced pancreatic and biliary cancer. Ann Oncol 7: 593-600.

38. Mitry E, Taïeb J, Artru P, Boige V, Vaillant JN, et al. (2004) Combination of folinic acid, 5-fluorouracil bolus and infusion, and cisplatin (LV5FU2-P regimen) in patients with advanced gastric or gastroesophageal junction carcinoma. Ann Oncol 15: 765-769.

39. Raderer M, Hejna MH, Valencak JB, Kornek GV, Weinländer GS et al.(1999) Two consecutive phase II studies of 5-fluorouracil/leucovorin/mitomycin C and of gemcitabine in patients with advanced biliary cancer. Oncology 56: 177-180.

40. Gebbia V, Giuliani F, Maiello E, Colucci G, Verderame F, et al. (2001) Treatment of inoperable and/or metastatic biliary tree carcinomas with single-agent gemcitabine or in combination with levofolinic acid and infusional fluorouracil: Results of a multicenter phase II study. J Clin Oncol 19: 4089-4091.

41. Penz M, Kornek GV, Raderer M, Ulrich-Pur H, Fiebiger W et al. (2001) Phase II trial of two-weekly gemcitabine in patients with advanced biliary tract cancer. Ann Oncol 12: 183-186.

42. He W, Wang B, Zhuang Y, Shao D, Sun K, Chen J (2012) Berberine Inhibits growth and induces G1 arrest and apoptosis in human cholangiocarcinoma QBC939 cells. J Pharmacol Sci 119(4): 341-348.

43. Saville MW, Lietzau J, Pluda JM, Feuerstein I, Odom J, et al. (1995) Treatment of HIV-associated Kaposi's sarcoma with paclitaxel. Lancet 346: 26-28.

44. Manegold C (2003) Pemetrexed (Alimta, MTA, multitargeted antifolate, LY231514) for malignant pleural mesothelioma. Semin Oncol 30: 32-36.

45. Cohen MH, Justice R, Pazdur R, Cortazar P (2009) Approval summary: pemetrexed in the initial treatment of advanced/metastatic non-small cell lung cancer. Oncologist 14: 930-935.

46. Rossi A, Ricciardi S, Maione P, de Marinis F, Gridelli C (2009) Pemetrexed in the treatment of advanced non-squamous lung cancer. Lung Cancer 66: 141-149.

47. Hass HG, Nehls O, Jobst J, Frilling A, Vogel U, Kaiser S (2008) Identification of osteopontin as the most consistently over-expressed gene in intrahepatic cholangiocarcinoma: detection by oligonucleotide microarray and real-time PCR analysis. World J Gastroenterol 14: 2501-2510.

48. Sulpice L, Rayar M, Desille M, Turlin B, Fautrel A, et al. (2013) Molecular profiling of stroma identifies osteopontin as an independent predictor of poor prognosis in intrahepatic cholangiocarcinoma. Hepatology 58: 1992-2000.

49. Shen DY, Zhan YH, Wang QM, Rui G, Zhang ZM (2013) Oncogenic potential of cyclin kinase subunit-2 in cholangiocarcinoma. Liver Int 33: 137-148.

50. Gibbs KD Jr, Jager A, Crespo O, Goltsev Y, Trejo A et al. (2012) Decoupling of tumor-initiating activity from stable immunophenotype in HoxA9-Meis1-driven AML. Cell Stem Cell 10: 210-217.

51. Dubrovsky L, Pankov D, Brea EJ, Dao T4, Scott A4 et al. (2014) A TCR-mimic antibody to WT1 bypasses tyrosine kinase inhibitor resistance in human BCR-ABL+ leukemias. Blood 123: 3296-3304.

52. Lilljebjorn H, Agerstam H, Orsmark-Pietras C, Rissler M, Ehrencrona H, et al. (2014) RNA-seq identifies clinically relevant fusion genes in leukemia including a novel MEF2D/CSF1R fusion responsive to imatinib. Leukemia 28: 977-979.