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REVIEW

- 420 Usefulness of endoscopic ultrasound-guided fine needle aspiration in the diagnosis of hepatic, gallbladder and biliary tract Lesions
Hammoud GM, Almashhrawi A, Ibdah JA

MINIREVIEWS

- 430 Vitamin D and colon cancer
Klampfer L

Contents

World Journal of Gastrointestinal Oncology
Volume 6 Number 11 November 15, 2014

APPENDIX I-V Instructions to authors

ABOUT COVER Editorial Board Member of *World Journal of Gastrointestinal Oncology*, Jamal A Ibdah, MD, PhD, AGAF, Professor, Director, Division of Gastroenterology and Hepatology, MU Institute for Clinical and Translational Science, Division of Gastroenterology and Hepatology, University of Missouri, One Hospital Drive, CE 405, DC043.00, Columbia, MO 65212, United States

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Usefulness of endoscopic ultrasound-guided fine needle aspiration in the diagnosis of hepatic, gallbladder and biliary tract Lesions

Ghassan M Hammoud, Ashraf Almashhrawi, Jamal A Ibdah

Ghassan M Hammoud, Ashraf Almashhrawi, Jamal A Ibdah, Division of Gastroenterology and Hepatology, University of Missouri, Columbia, MO 65212, United States

Author contributions: Hammoud GM and Almashhrawi A wrote the manuscript; Ibdah JA edited and revised the manuscript.

Correspondence to: Jamal A Ibdah, MD, PhD, Professor and Director, Division of Gastroenterology and Hepatology, University of Missouri-Columbia, 230 Jesse Hall, Columbia, MO 65212, United States. ibdahj@health.missouri.edu

Telephone: +1-573-8820482 Fax: +1-573-8844595

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Abstract

Endoscopic ultrasound (EUS)-guided fine needle aspiration (FNA) of the liver is a safe procedure in the diagnosis and staging of hepatobiliary malignancies with a minimal major complication rate. EUS-FNA is useful for liver lesions poorly accessible to other imaging modalities of the liver. EUS-guided FNA of biliary neoplasia and malignant biliary stricture is superior to the conventional endoscopic brushing and biopsy.

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Key words: Endoscopic ultrasound; Fine needle aspiration; Hepatocellular carcinoma; Bile duct stricture; Gallbladder; Cholangiocarcinoma; Biliary drainage

Core tip: The present article reviews the usefulness of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) in patients with focal liver and biliary tract lesions. We conducted MEDLINE search using the terms "endoscopic ultrasound-guided fine needle aspiration", "focal liver lesions" and "biliary tract lesions", "EUS and biliary stricture", "EUS and focal liver mass", "EUS and

cholangiocarcinoma" and "EUS and gallbladder" to retrieve articles published between 1999 to 2014.

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INTRODUCTION

Endoscopic ultrasonography (EUS) has become an indispensable diagnostic and therapeutic procedure in the field of gastroenterology coupling endoscopy with high frequency echo sonography. Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is performed using the curved linear array echoendoscope (Figure 1) using various needles (Figure 2). The recently introduced forward viewing linear echoendoscope is gaining momentum in endoscopic ultrasound-guided interventions (Figure 1). EUS-FNA is minimally invasive that is utilized for procurement of tissue from unresectable tumors. EUS-guided fine needle aspiration is used increasingly for the diagnosis of mediastinal, pancreatic and gastric tumors, however, not much is known about EUS-FNA in hepatic lesions. EUS imaging of the liver is currently limited to the left lobe, the proximal right lobe, the hilum and part of the intrahepatic biliary tract. EUS-FNA may be considered as an alternative to liver percutaneous biopsy in patients at high risk of bleeding or with small lesions of the liver uncharacterized by cross-sectional abdominal imaging. EUS-guided biliary drainage (EUS-BD) was developed using a curved linear array echoendoscope for cases with failed endoscopic biliary drainage. Table 1 summarizes the use of endoscopic ultrasound-guided

Table 1 Summary of the use of endoscopic ultrasound-guided fine needle aspiration in the diagnosis of hepatic and biliary tract lesions

Diagnosis of focal malignant and benign liver lesions
Diagnosis of malignant biliary stricture and neoplasia
Preoperative staging of hepatocellular carcinoma and lymph node metastasis
Ablation of focal malignant and benign liver lesions
Liver biopsy
Fluid acquisition and biopsy of peritoneal and omental deposits
Drainage of intrahepatic and extrahepatic biliary tree
Drainage of hepatic abscesses

fine needle aspiration in the diagnosis and management of hepatic, gallbladder and biliary tract lesions.

FEASIBILITY OF ENDOSCOPIC ULTRASOUND-GUIDED FINE NEEDLE ASPIRATION OF FOCAL LIVER LESIONS

Focal liver lesions include simple liver cyst, focal nodular hyperplasia, hepatic adenoma, hepatic hemangioma, regenerative nodular hyperplasia, biliary cystadenoma, intrahepatic cholangiocarcinoma, hepatocellular carcinoma and metastatic liver lesions. The majority of these lesions can be diagnosed with certainty by cross-sectional abdominal imaging and by percutaneous liver biopsy. However, small lesions less than 1-cm in diameter may not be well characterized by abdominal ultrasound (US), computed tomography (CT) and/or magnetic resonance imaging (MRI). In general, the lowest ultrasound frequency available should be used to maximize penetration. EUS-guided liver biopsy using a 19-gauge FNA needle (non-Trucut) and EUS-guided Trucut needle appear to be feasible, safe and provide excellent diagnostic yield and specimen adequacy^[1-3]. In a retrospective study by DeWitt *et al*^[4], EUS-FNA of liver lesions that range from 3-40 mm in size was performed in 77 patients^[4]. Of these lesions, 58% were diagnostic for malignancy, 33% were benign, and 9% were nondiagnostic. In a study by tenBerge *et al*^[5], EUS-FNA was used to sample liver lesions in 167 patients. The indications were pancreatic mass in 37%, liver metastasis of unknown origin in 20%, esophageal, gastric and liver masses. EUS-FNA of the liver revealed malignancy in patients when abdominal ultrasonography-guided FNA and CT-guided FNA have failed. Crowe *et al*^[6] compared 34 percutaneous computerized tomographic-guided fine needle aspiration liver biopsies and 16 EUS-FNA liver biopsies showed comparable results. These studies and others suggest that EUS-FNA is feasible and comparable to US/CT-guided biopsy in the diagnosis of patients with focal liver lesions.

Malignant focal/metastatic liver lesions

EUS can provide high resolution imaging of the left hepatic lobe to detect unsuspected metastatic disease during staging and may deter from unnecessary surgery^[7,8]. EUS-FNA of liver lesions can provide useful information for future management. Hepatic metastasis is generally echo-

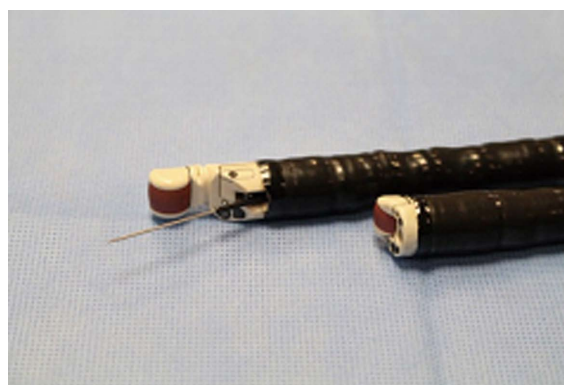


Figure 1 The curved linear array videoechoendoscope (GF-UCT180) (Back); The new prototype forward viewing linear array videoechoendoscope (TGF-UC180J) (Front).



Figure 2 Various echoendoscopic needles used for fine needle aspiration.

poor without a distinct border such as the one seen in pancreatic and colon metastasis (Figure 3) or echo-rich such as seen in metastatic neuroendocrine tumors and renal cell carcinoma (Figure 4). EUS-FNA can detect tumors less than 3 mm in size^[7]. Solid liver lesions accessible by EUS may be safely sampled by EUS-FNA. The use of stylet during FNA does not appear to confer any advantage with regards to the adequacy of specimen or diagnostic yield of malignancy^[9]. In a prospective study of 132 subjects with newly diagnosed tumors, the diagnostic accuracy of EUS/EUS-FNA and CT scan in detecting hepatic metastasis was 98% and 92%, respectively ($P = 0.0578$)^[10]. In a large single-center experience, the sensitivity of EUS-FNA for the diagnosis of liver cancer ranged from 82% to 94%^[4]. In a prospective study of 41 patients, 33 of whom had clinical findings suggestive of liver malignancies, EUS-FNA provided biopsy specimens in 40/41 patients^[11]. Combining histological and cytological features had a sensitivity of 94%, specificity of 100%, negative predictive value of 78%, and positive predictive value of 100%^[11]. These data suggest that EUS-FNA is a sensitive diagnostic procedure in patients with focal malignant liver lesions especially to those confined to left hepatic lobe.

Hepatocellular carcinoma

EUS-FNA may be useful in the diagnosis of focal liver lesions, early hepatocellular carcinoma, and evaluation of

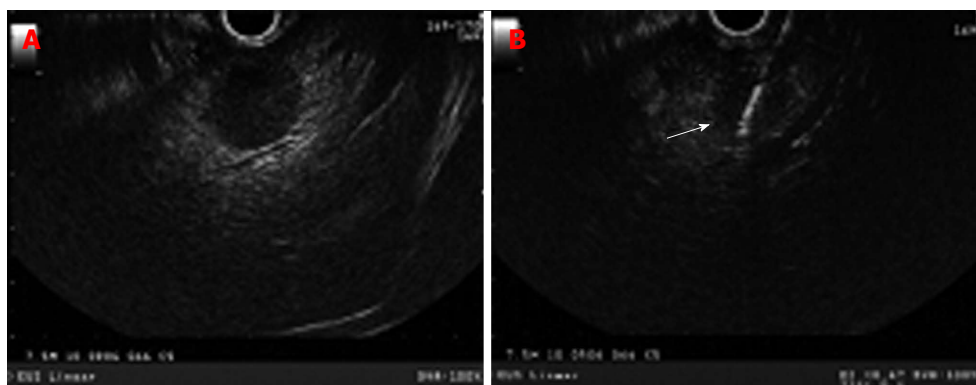


Figure 3 Curved linear echoendoscope showing a rounded hypoechoic left lobe liver lesion with no well-defined border representing liver metastasis in a patient with pancreatic adenocarcinoma (A); fine needle aspiration (white arrow) was performed using 22 gauge needle (B).

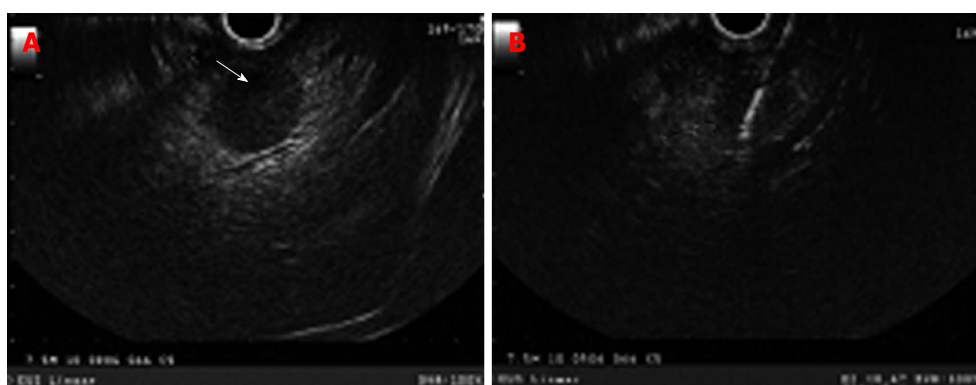


Figure 4 Hyperechoic rounded liver lesion (white arrow) representing a metastasis in patient with pancreatic neuroendocrine tumor with biliary obstruction and dilated intrahepatic duct (A); endoscopic ultrasound-guided fine needle aspiration of liver lesion using 22 gauge needle (B).

perihepatic adenopathy^[12-15]. Hepatocellular carcinoma (HCC) may appear on EUS images either as hypoechoic or hyperechoic^[16]. Burrell *et al.*^[17] showed that lesions smaller than 1cm in diameter are missed in a significant percentage (70%) of the patients by modalities such as CT imaging^[14,18] and magnetic resonance imaging^[18]. EUS and EUS-FNA are particularly valuable for the preoperative staging of hepatocellular and metastatic liver carcinoma. In a study by Awad *et al.*^[18], EUS identified liver lesions 0.3-14 cm in size in all 14 study patients with hepatocellular cancer and metastatic lesions who underwent both dynamic CT scans and EUS^[18]. Moreover, in 28% of the patients, EUS identified new lesions less than 0.5 cm in size. In a prospective single-center study evaluating 17 patients who underwent cross-sectional imaging and EUS, 9 had liver tumors^[16]. EUS-FNA established a tissue diagnosis in 8 of the 9 cases. The diagnostic accuracy of transabdominal ultrasonography, abdominal CT, MRI, and EUS/EUS-FNA were 38%, 69%, 92%, and 94%, respectively^[16]. Another retrospective study evaluated the sensitivity and complications of EUS-FNA of liver nodules in 14 patients, performed by single endoscopist^[19]. Twenty-one percent of the cases were hepatocellular carcinoma. The sensitivity of diagnosis of malignant liver lesions utilizing cytology was 78.5%. However, combining clinical course and pathology increased the sensitivity to 100%. These data suggest that EUS has an excellent

diagnostic accuracy in patients with HCC.

Moreover, EUS-guided fine needle aspiration of portal vein thrombus to detect malignancy has been described in literature^[20,21]. More recently, a newly developed promising technique utilizing real time-sonoelastography (RTE) by EUS might improve the characterization and differentiation between benign and malignant focal liver lesions^[22].

Screening and treatment of HCC

The use of EUS-FNA in screening for HCC is limited by the semi-invasive nature of the procedure as well as its inability to evaluate all liver segments at this time^[13]. Nevertheless, EUS can provide an additional option for treatment in patients with hepatocellular carcinoma who are difficult to treat utilizing percutaneous ablative therapy such as endoscopic ultrasound-guided ethanol injection^[23,24] and EUS-guided Nd:YAG laser ablation of a caudate lobe hepatocellular carcinoma^[25].

Benign focal liver lesions

Large hepatic cysts are amenable to percutaneous drainage or surgical resection. EUS-guided ethanol injection has been shown to be effective in treating patients with large hepatic cysts especially in the left hepatic lobe. In a retrospective study evaluating 17 patients with 19 hepatic cysts (median cyst volume before therapy was 368.9 mL)^[26], ten cysts were drained by the percutaneous

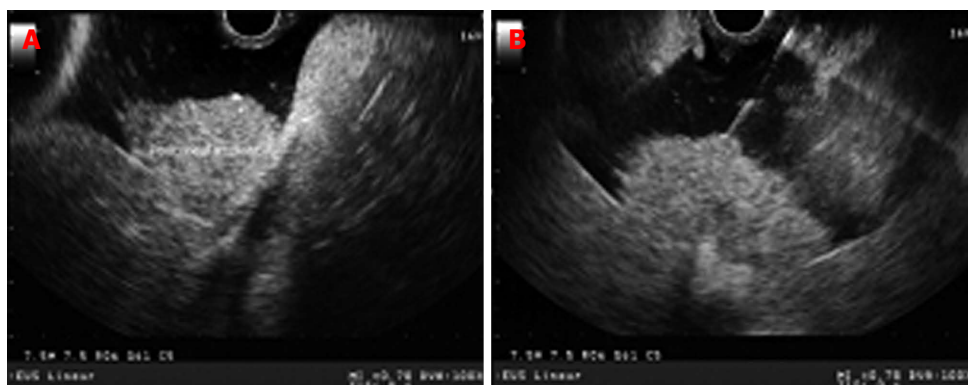


Figure 5 Peritoneal deposits in a patient with malignant ascites. Peritoneal implants appear as hypoechoic in comparison to the surrounding tissue but hyperechoic in comparison to the anechoic ascitic fluid (A); endoscopic ultrasound-guided fine needle aspiration of a large peritoneal deposit (B).

approach and 8 cysts underwent EUS-guided aspiration and lavage treatment. During 15-mo follow-up, the cysts showed nearly 100% reduction in the EUS-guided group compared to 97% reduction in the percutaneous group. Furthermore, EUS-FNA has also shown excellent success rates in selected patients with hepatic abscesses. In a recent review of the literature by Singhal *et al*^[27], seven studies have reported 100% technical and clinical success rates of EUS-guided drainage of hepatic abscesses in patients refractory or not amenable to percutaneous drainage.

Ascites and peritoneal metastasis

EUS-guided paracentesis is valuable in the cytologic diagnosis and staging of malignant ascites^[28,29]. EUS frequently identifies ascites missed by other imaging modalities and may identify malignancy^[30]. It is particularly useful when CT imaging does not identify abnormalities^[31]. EUS-FNA can be performed safely for therapeutic paracentesis^[32]. In a retrospective single center study that evaluated 101 patients who underwent EUS-guided paracentesis, the specificity, sensitivity, positive and negative predictive values, and diagnostic accuracy were 100%, 80%, 100%, 95% and 96%, respectively^[29]. Furthermore, EUS-FNA can be used effectively and safely to obtain tissue from the peritoneum for diagnosis of tuberculous peritonitis^[33]. EUS-FNA allows the sampling of peritoneal metastatic lesions, which appear on EUS images as hyperechoic compared to surrounding anechoic ascitic fluid (Figure 5). In a small study involving 12 patients with undiagnosed ascites, peritoneal deposits noted in 10 (83.3%) patients^[34]. The cytological results were positive for malignancy in 6 of those patients, while the remaining four patients had inflammatory cells.

ENDOSCOPIC ULTRASOUND-GUIDED FINE NEEDLE ASPIRATION OF BILE DUCT, GALLBLADDER AND AMPULLARY LESIONS

Cholangiocarcinoma and proximal biliary strictures

Preoperative tissue diagnosis is required for hilar neoplasia

[cholangiocarcinoma (CCA)] to avoid risk of unnecessary extensive surgery. Endoscopic transpapillary brush cytology and forceps biopsy are used for the pathological diagnosis of malignant biliary strictures. Endoscopic retrograde cholangiography (ERC) is currently the main diagnostic procedure performed to obtain sampling of the biliary tree. However, the sensitivity and specificity of obtaining a sample in biliary neoplasia is variable. EUS is capable of visualizing the hilum at the duodenal bulb by tracing the common bile duct (CBD) towards the liver hilum. In a meta-analysis of 36 studies by Garrow *et al*^[35] EUS has a sensitivity of 78% and specificity of 84% in detecting malignant biliary strictures. Nayar *et al*^[36] reported on 32 patients who underwent 36 procedures for hilar lesions. The overall sensitivity, accuracy, specificity, positive predictive value and negative predictive value of EUS-FNA were 52%, 68%, 100%, 100% and 54%, respectively. Fritscher-Ravens *et al*^[37] prospectively evaluated 44 patients with hilar strictures diagnosed by CT and/or Endoscopic retrograde cholangiopancreatography (ERCP) that were suspicious for hilar cholangiocarcinoma but had inconclusive tissue diagnosis. The sensitivity, accuracy, and specificity of EUS-FNA in this study were 89%, 91%, and 100%, respectively. Moreover, EUS and EUS-FNA changed preplanned surgical approach in about half of these patients^[37]. The above studies suggest that hilar neoplasia can be sampled by EUS-FNA although the accuracy and sensitivity were not robust. Moreover, EUS-FNA may be considered in evaluating regional lymph nodes to evaluate for metastasis in patients with unresectable hilar cholangiocarcinoma^[38,39]. EUS-FNA in patients with cholangiocarcinoma did not appear to adversely affect the overall survival^[40].

Distal malignant biliary stricture

The sensitivity of EUS-FNA is much higher in distal malignant biliary strictures than proximal strictures. Malignant distal biliary strictures are most commonly secondary to pancreatic malignancy and/or distal bile duct cholangiocarcinoma (Figure 6). In a recent prospective comparative one-year study of 51 patients who underwent EUS and ERCP in the same session for evaluation of malignant biliary obstruction^[41], EUS-FNA was supe-



Figure 6 Malignant distal biliary strictures are most commonly secondary to pancreatic malignancy and/or distal bile duct cholangiocarcinoma. A: Distal common bile duct stricture secondary to a large heterogeneous hypoechoic pancreas head mass with irregular border; B: Endoscopic ultrasound-guided fine needle aspiration of pancreas head mass/stricture; C: Distal irregular common bile duct stricture seen on cholangiogram.

rior to ERCP in tissue sampling for evaluating suspected malignant biliary obstruction, especially for pancreatic masses with an overall accuracy and sensitivity of 94% and 94% for EUS-FNA, and 53% and 50% for ERCP sampling, respectively. In an observation study of prospectively collected data of 228 patients with biliary strictures who underwent EUS^[42]. Cholangiocarcinoma was detected in eighty-one. Fifty-one of the patients (63%) had distal and 30 (37%) had proximal CCA. The overall sensitivity of EUS-FNA for the diagnosis of CCA was 73% and was significantly higher in distal compared to proximal CCA (81% *vs* 59%, respectively; $P = 0.04$). Furthermore, a retrospective analysis of 342 patients who underwent EUS-FNA after presenting with biliary stricture and obstructive jaundice^[43] showed an overall 92.4% accuracy of EUS-FNA for diagnosing malignancy with 91.5% sensitivity and 80.9% negative predictive value. These studies and others demonstrate the higher sensitivity of EUS-FNA in distal biliary stricture. Moreover, EUS-FNA appears equivalent to ERCP sampling for biliary tumors and indeterminate strictures^[41] and may provide a diagnosis of malignancy when ERCP sampling is indeterminate^[44]. Moreover, EUS-FNA can have a role in diagnosing other lesions that may mimic cholangiocarcinoma and present either as a mass or with obstructive jaundice. Such lesions as epithelial *vs* nonepithelial tumors, neuroendocrine tumors, lymphoma, and metastasis from other primaries^[45,46].

Endoscopic ultrasound-guided biliary access/drainage

ERCP is currently the standard of care for biliary drainage, however the failed cannulation rates ranges 3% to 5% in experienced hands. EUS-guided biliary drainage includes EUS-guided choledochoduodenostomy^[47], hepaticogastrostomy^[48], and EUS-guided transpapillary rendezvous biliary drainage^[49]. The procedure technique has been described as follows^[50]: the linear-array EUS scope is placed against the cardia or lesser curve of the stomach in a patient with dilated left intrahepatic biliary tree for hepaticogastrostomy or against the bulb of the duodenum for choledochoduodenostomy. The dilated bile duct or left intrahepatic duct which appears as hyperechoic structure running alongside the portal venous system

without Doppler flow signals is then identified and punctured using a 19-gauge or 22-gauge needle. The stylet is then removed followed by contrast injection to visualize the biliary tree under fluoroscopy. A 0.035" or 0.021" guidewire is subsequently passed via the FNA needle into the bile duct or dilated intrahepatic duct. The needle knife is then used to make an incision of the gastric or duodenal wall under EUS guidance for preparation of dilation of the transmural tract. Dilation can be performed using 4.5F to 5F ERCP cannula, 4-mm or 6-mm dilating biliary balloon. A plastic biliary stent or self-expandable fully covered metal stent can then be placed^[51,52]. In a large multicenter, nonrandomized retrospective study of 240 patients who underwent EUS-guided bile duct access and drainage^[53], success was achieved in 87% of the cases. Similarly, in extrahepatic and intrahepatic approaches, the success rate was 84.3% *vs* 90.4%; respectively.

Gallbladder lesions

EUS-FNA has gained momentum in sampling gallbladder masses for diagnostic and staging purposes with accuracy reaching 100% in early stages. Sadamoto *et al*^[54] reported EUS accuracy of 100% for in situ tumors (Tis), 76% for T1, 85% for T2, and 93% for T3 and T4 lesions. In one series, EUS-FNA provided accurate diagnosis of six patients with obstructive jaundice (five with gallbladder adenocarcinomas) where CT scans mostly failed to detect the causing lesions^[55]. Jacobson *et al*^[56] described similar findings in four out of five patients diagnosed with adenocarcinoma of the gallbladder. Meara *et al*^[57] reported sensitivity of 80% and specificity of 100% in diagnosing gallbladder wall lesions.

EUS and transabdominal US are usually viewed as good tools to evaluate gallbladder polyps with superior sensitivities for EUS 97% *vs* transabdominal US 71% in one study^[58]. Diagnostic distinction between malignant and non-malignant polyps for the purpose of staging and determining next steps management, remains mostly dependent on the ultrasonographic features of the polyps rather than tissue sampling^[54]. No reports of the use of EUS-FNA in approaching gallbladder polyps were found. Endoscopic ultrasound-guided transmural gallbladder drainage with placement of self-expandable stent has

Table 2 Summary of the sensitivity, specificity and diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration in the diagnosis of focal hepatic, gallbladder and biliary tract lesions

Study, year, number	Sensitivity (%)	Specificity (%)	Diagnostic accuracy (%)
Focal malignant liver lesions			
DeWitt <i>et al</i> ^[41] , 2003, <i>n</i> = 77	82-94	-	-
Hollerbach <i>et al</i> ^[111] , 2003, <i>n</i> = 44	94	100	-
Singh <i>et al</i> ^[16] , 2007, <i>n</i> = 17	89	100	94
	CT (71)	67	69
Prachayakul <i>et al</i> ^[139] , 2012, <i>n</i> = 14	MR (86)	100	92
	78.5	-	-
Malignant biliary tract and gallbladder lesions			
Garrow <i>et al</i> ^[35] , 2007, 36 studies, <i>n</i> = 3532	78	84	90
Nayar <i>et al</i> ^[36] , 2011, <i>n</i> = 32	52	100	68
Fritscher-Ravens <i>et al</i> ^[37] , 2004, <i>n</i> = 44	89	100	91
Weilert <i>et al</i> ^[41] , 2014, <i>n</i> = 51	94	100	94
	ERCP	100	53
Mohamadnejad <i>et al</i> ^[42] , 2011, <i>n</i> = 228	brushing (50)	-	-
	73	-	-
Tummala <i>et al</i> ^[43] , 2013, <i>n</i> = 342	ERCP	-	-
	brushing (27)	-	-
Meara <i>et al</i> ^[57] , 2006, <i>n</i> = 53	91.5	-	92.4
	80	100	-
	ERCP	75	-
	brushing (13)	-	-

CT: Computed tomography; MR: Magnetic resonance; ERCP: Endoscopic retrograde cholangiopancreatography.

been reported and is technically successful for the management of acute cholecystitis in high risk patients^[59-61]. Table 2 summarizes the sensitivity, specificity and diagnostic accuracy in the reported studies.

Ampullary tumors

EUS-FNA can provide an excellent diagnostic accuracy in distinguishing between benign and malignant ampullary tumors in comparison to surface biopsy with duodenoscopy, and/or intra-ampullary biopsy, and/or brush cytology with ERCP, and/or intra-ampullary biopsy after endoscopic sphincterotomy (100% *vs* 70%)^[62]. Furthermore, the diagnostic accuracy of EUS-FNA for ampullary tumors supersedes that without EUS-FNA. In a retrospective study by Roberts *et al*^[63], rates of diagnostic accuracy in high-grade dysplasia, low-grade dysplasia, and adenocarcinoma were 20%, 72%, and 96%, respectively, in the non-EUS group, and 50%, 93%, and 100%, respectively, in the EUS group.

ENDOSCOPIC ULTRASOUND AND RAPID ON-SITE CYTOLOGY EVALUATION

The diagnostic accuracy of EUS-FNA is dependent on how the sample is processed after acquisition. The pres-

ence of a rapid on-site cytology evaluation (ROSE) by a cytopathologist in the vicinity where the sample is obtained has been shown to improve the diagnostic yield of the procedure^[64]. ROSE may allow a less number of needle passes and ensure adequacy of the sample obtained by onsite staining prior to completion of procedure. In general, the diagnostic yield of EUS-FNA with ROSE in most studies exceeds 90%. Meara *et al*^[57] reported on 53 cases undergone EUS-FNA from 46 bile duct and seven gallbladder lesions where ROSE was available. All cases initially diagnosed as suspicious/malignant were confirmed on the final cytological interpretation. The specificity for EUS-FNA was 100% with sensitivity rates of 80% and 87% from clinically suspected malignancies of gallbladder and biliary tract, respectively. A retrospective study by Jhala *et al*^[65] provided on-site diagnosis of malignancy on 485 EUS-FNA of the pancreas (*n* = 305), lymph nodes (*n* = 91), biliary tree (*n* = 47), liver (*n* = 15), gastrointestinal tract (*n* = 19), and adrenal gland (*n* = 8). A significantly higher degree of concordance was noted for unequivocal diagnosis of malignancy *vs* no malignancy (98.9% *vs* 67.2%) between on-site and final cytologic diagnosis. These studies have demonstrated ROSE by cytopathologist and interpretation significantly improves the diagnostic yield of EUS-FNA.

COMPLICATIONS OF ENDOSCOPIC ULTRASOUND-GUIDED FINE NEEDLE ASPIRATION OF HEPATIC AND BILE DUCT LESIONS

In a retrospective questionnaire sent to 130 EUS-FNA centers across the world^[5], 167 cases of EUS-FNA of the liver were reported by 21 centers. A complication was reported in 6 (4%) of the 167 cases including the following: death in 1 patient, bleeding (1), fever (2), and pain (2)^[5]. EUS-guided liver biopsy appears to be safe and associated with no significant complications^[2-4,66]. Several studies have reported no adverse events related to EUS-FNA of bile duct strictures, gallbladder and masses^[41,42,56,57,67]. However, EUS-FNA of malignant biliary lesions was reported to have a risk of bleeding, infection, or pancreatitis in less than 2% of the cases^[68]. Hemobilia was reported in 1.3% of patients who underwent EUS-FNA of malignant biliary stricture^[42]. Bacteremia after EUS-FNA is rare. However, prophylactic antibiotics should be given prior and after EUS-FNA of biliary tract in patients with biliary obstruction. EUS-guided diagnostic abdominal paracentesis was not associated with any complication in one study^[28]. Bile peritonitis has been reported after inadvertent biliary puncture during EUS-FNA^[69]. Complications of EUS-guided biliary drainage included pneumoperitoneum 5%, bleeding 11%, bile leak/peritonitis 10%, and cholangitis 5%^[53]. Needle track tumor seeding has been reported and is a risk after EUS-FNA of malignant biliary neoplasia^[70,71]. EUS-FNA of malignant biliary stricture is considered a contraindication in patients eligible for liver transplantation. Cholecystitis and bile peri-

tonitis have been reported after EUS-FNA of gallbladder lesions^[72]. Bleeding after EUS-FNA of solid tumor is rare and appears as an expanding extraluminal echopoor region adjacent to the sampled lesion^[73].

LIMITATIONS OF ENDOSCOPIC ULTRASOUND-GUIDED FINE NEEDLE ASPIRATION OF HEPATIC AND BILE DUCT LESIONS

The head of pancreas and CBD are not visualized after Roux-en-Y surgery and Billroth II surgery if the afferent limb is not intubated. Presence of vascular structures or collaterals in needle path may limit EUS-FNA of focal lesions. Because the right liver lobe is farther away from the probe, it is generally not seen except in small parts. The presence of pneumobilia, fatty infiltration, calcifications and extensive fibrosis may interfere with ultrasound beam and images. Endosonographer's experience, time consumed to image the liver and patient's body habitus are of critical importance to clearly identify and diagnose focal liver lesions. The miss rate for resectable pancreaticobiliary malignancy by EUS-FNA is rather small. Moreover, EUS and EUS-FNA may not be widely available and require an expertise with dedicated echosonographer in the field. With improving resolution and widespread use of EUS with dedicated formal training, small liver metastasis and other focal liver lesions are being increasingly detected. EUS does not use intravenous contrast to evaluate the nature of focal liver lesions and thus correlation with other cross-sectional imaging such as CT and/or MR is needed. However, the technology has dramatically improved. The use of color and power Doppler imaging, three-dimensional imaging, electronic scanning, tissue harmonic imaging, elastography, and recently contrast-enhanced images have improved the diagnostic capability. The depth of tumor infiltration and differentiation between infiltrating or exophytic lesions can now be assessed with greater accuracy^[74-76].

CONCLUSION

Endoscopic ultrasound-guided fine needle aspiration of the liver, gallbladder and biliary tract is feasible and provides an excellent diagnostic accuracy. The presence of ROSE has increased the diagnostic yield. EUS-FNA is capable to differentiate between focal benign or malignant liver lesions. The widespread of EUS and increase formal training have enhanced the diagnostic and therapeutic armamentarium of EUS in hepatobiliary disorders. EUS-FNA should be considered as an adjunct to other cross-sectional imaging in the differentiation between benign and focal hepatobiliary disorders. EUS-guided interventions such as fine-needle injections, tumor ablative therapies and biliary drainage have increased the application of EUS and is considered as an adjunct to other modalities.

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Vitamin D and colon cancer

Lidija Klampfer

Lidija Klampfer, Southern Research Institute, Birmingham, AL 35205, United States

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Correspondence to: Lidija Klampfer, PhD, Southern Research Institute, 2000 9th Avenue, Birmingham, AL 35205,

United States. klampfer@southernresearch.org

Telephone: +1-205-5812731

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Core tip: Epidemiological studies suggest that deficiency of vitamin D increases the incidence of colon cancer and also has a negative impact on the survival of colon cancer patients. The ability of 1,25D₃ to interfere with Wnt signaling and to ameliorate inflammation is likely to contribute to its anticancer activity.

Klampfer L. Vitamin D and colon cancer. *World J Gastrointest Oncol* 2014; 6(11): 430-437 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v6/i11/430.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v6.i11.430>

Abstract

Calcitriol, 1 α , 25-dihydroxyvitamin D₃ (1,25 (OH)₂D₃), the most active form of vitamin D, is a pleiotropic hormone with a wide range of biological activities. Due to its ability to regulate calcium and phosphate metabolism, 1,25D₃ plays a major role in bone health. In addition, 1,25D₃ binds to the vitamin D receptor and thereby regulates the expression of a number of genes which control growth, differentiation and survival of cancer cells. In agreement, the levels of vitamin D₃ appear to be an essential determinant for the development and progression of colon cancer and supplementation with vitamin D₃ is effective in suppressing intestinal tumorigenesis in animal models. Vitamin D₃ has been estimated to lower the incidence of colorectal cancer by 50%, which is consistent with the inverse correlation between dietary vitamin D₃ intake or sunlight exposure and human colorectal cancer. Several studies confirmed that increasing vitamin D₃ lowers colon cancer incidence, reduces polyp recurrence, and that sufficient levels of vitamin D₃ are associated with better overall survival of colon cancer patients. Vitamin D regulates the homeostasis of intestinal epithelium by modulating the oncogenic Wnt signaling pathway and by inhibiting tumor-promoting inflammation. Both activities contribute to the ability of 1,25D₃ to prevent the development and progression of colon cancer.

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INTRODUCTION

The biologically active form of vitamin D₃, 1 α ,25(OH)₂D₃ (1,25D₃), is obtained by 25-hydroxylation of vitamin D₃ in the liver and 1 α -hydroxylation in the kidney, liver or other tissues. Hydroxylation of 25(OH)D₃ by CYP27B1 yields the hormonally active form 1,25(OH)₂D₃, which is metabolized to less active metabolites by CYP24A1 (reviewed in^[1]). While the levels of CYP21B1 have been shown to be reduced in some cancers, the levels of CYP24A1 are increased in cancer cells, which may contribute to the resistance of some tumors to 1,25D₃^[2].

1,25D₃ exerts most of its biological activity through binding to a specific vitamin D₃ receptor (VDR), a member of the nuclear receptor superfamily^[1]. VDR binds to retinoid X receptor (RXR), and the VDR-RXR heterodimers bind to a vitamin D response element (VDRE), activating or repressing gene expression, which contribute to the anti-neoplastic activity of vitamin D. VDR associates with other transcription factors, such as SP1 and β -catenin^[3] and thereby also regulates the expression of genes that do not harbor the consensus VDRE. A number of cancer cell lines, including colon cancer cell lines tested in our laboratory, display a limited response to vitamin D₃ *in vitro*^[4] and the expression of VDR is

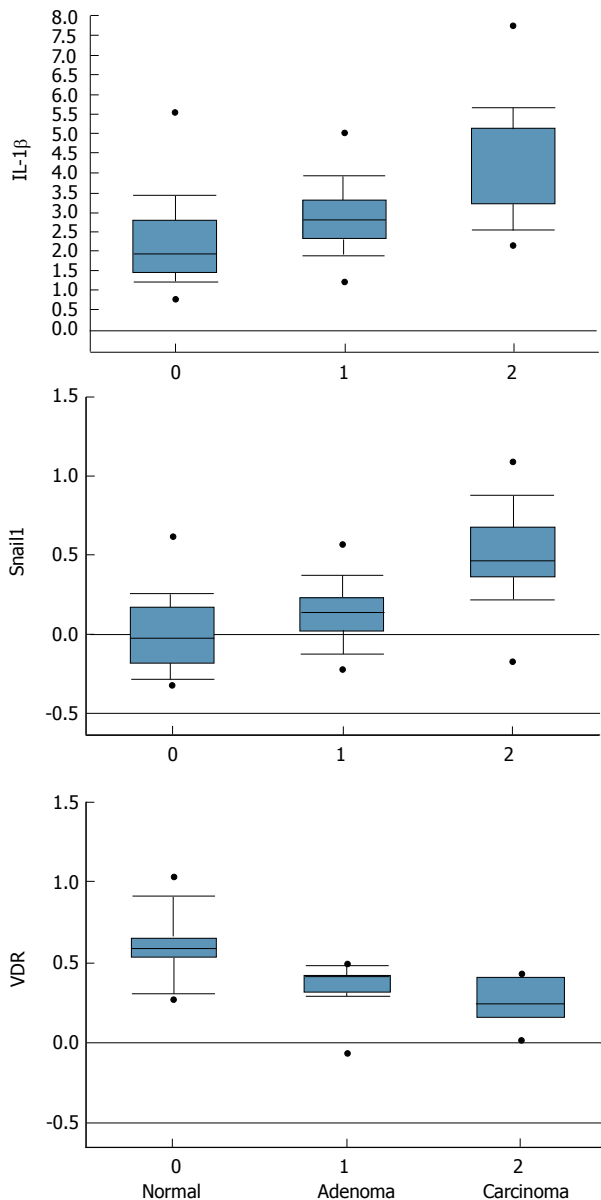


Figure 1 The expression levels of IL-1 β and Snail are increased and the levels of vitamin D receptor decreased in colon cancer patients (Skrypiack, *PLOS ONE* 2010^[71]). VDR: Vitamin D receptor.

downregulated in late stages of colon cancer^[5] (Figure 1), suggesting that vitamin D₃ may exert some of its biological activities in a VDR-independent manner, or that it targets cells in the tumor microenvironment. VDR^{-/-} mice display hyper-proliferation and have elevated levels of c-myc in both skin and colon, and VDR suppresses c-myc expression *in vitro* and *in vivo* in the absence of 1,25D₃^[6]. However, 1,25D₃ triggers association of VDR with c-myc and thereby promotes turnover of c-myc protein^[6], indicating that vitamin D signaling suppresses transcription of c-myc and also inhibits c-myc stability. In addition to its ability to inhibit c-Myc, 1,25D₃ induces the expression of its antagonist Mxd1/Mad1, suggesting that 1,25D₃ can exert its chemopreventive activity through regulation of the c-myc/Mxd1 network^[6].

The focus of this report is to discuss the role of vi-

tamin D in colon cancer, however the beneficial effects of vitamin D have been noted in other malignancies. Reduced serum levels of vitamin D were found in stage IV melanoma patients and it has been shown that melanoma patients with low serum levels of vitamin D developed metastasis earlier than patients with high levels of vitamin D^[7]. Similarly, chemopreventive activity of vitamin D has been observed in breast, ovarian, pancreatic and prostate cancer patients^[8].

In addition to its chemopreventive activity, 1,25D₃ or its analogues have been tested for their ability to improve the response to anticancer agents. Vitamin D and its derivatives have been shown to enhance the anticancer activity of 5FU, irinotecan and oxaliplatin both *in vitro* and *in vivo*^[9,10]. Although the therapeutic use of 1,25D₃ is restricted by its hypercalcemic activity, several 1,25D₃ analogues that retain the antitumor activity while being devoid of hypercalcemic effects, are currently being tested in clinical trials for a variety of malignancies.

VITAMIN D AND COLON CANCER

Recent case-controlled studies have established that there is an inverse correlation between serum levels of vitamin D and the incidence of polyps and adenomas in the colon^[11-13], consistent with the inverse correlation between dietary vitamin D₃ intake or sunlight exposure and human colorectal cancer^[14-17]. This is significant because a large segment of the human population suffers from vitamin D₃ insufficiency or deficiency^[18], which is particularly prevalent among colon cancer patients. Indeed, numerous studies have suggested that higher vitamin D₃ levels are associated with lower colon cancer incidence, reduced polyp recurrence and better overall survival of colon cancer patients^[19-22].

Vitamin D and its analogues reduce the growth of colon cancer xenografts and inhibit tumorigenesis in several genetic models of intestinal cancer. In agreement, dietary initiation of colon cancer in rodents, a model of sporadic colon cancer, has been shown to be prevented by supplementation with vitamin D₃ and Ca^[23,24].

Despite the established chemopreventive activity of vitamin D₃, its targets and the molecular basis for its antitumor activity remain poorly understood. 1,25D₃ inhibits growth of tumor cells by inducing the expression of cyclin-dependent kinase inhibitors, such as p21, p27, and cystatin D, and by inhibiting the expression of pro-proliferative genes, including c-myc and cyclin D1. In addition, 1,25D₃ has been shown to upregulate miR-627, which targets the histone demethylase jumonji domain containing protein 1A, and thereby inhibits proliferation of colon cancer cells *in vitro* and *in vivo* through epigenetic regulation^[25]. By increasing the expression of alkaline phosphatase, maltase, E-cadherin and cell adhesion proteins, vitamin D promotes differentiation. In a cell-type specific manner, vitamin D promotes apoptosis by regulating the expression of B-cell lymphoma 2 family members. Thus, due to its ability to affect multiple signaling pathways and to regulate many target genes, 1,25D₃

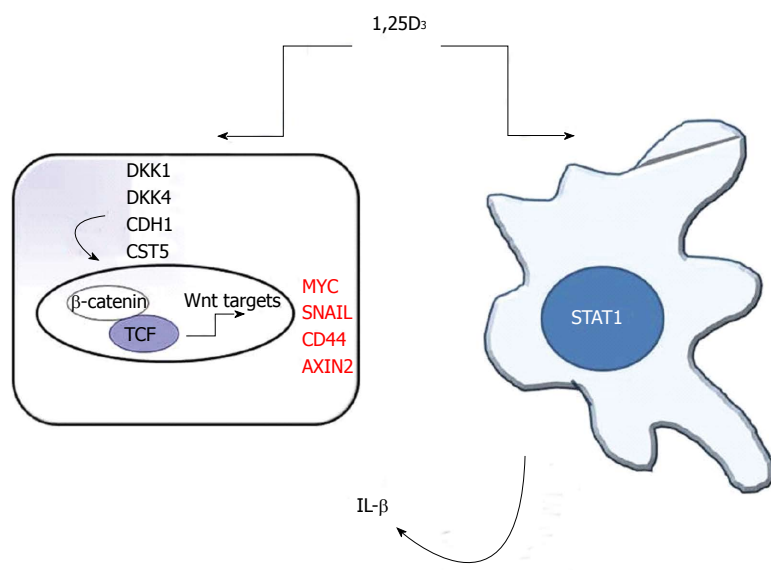


Figure 2 The multiple mechanisms whereby vitamin D inhibits Wnt signaling: 1,25D₃ acts on both tumor cells and tumor-associated macrophages (and potentially on other stromal cells). In tumor cells, 1,25D₃ promotes VDR/β-catenin binding and thus inhibits nuclear translocation of β-catenin. It also induces the expression of E-cadherin (CDH1), Dickkopf1 (DKK1), Dickkopf4 (DKK4) and cystatin 5 (CST5), antagonizing β-catenin/TCF transcriptional activity. As a result, the expression of several Wnt target genes, such as Snail, CD44, Myc, Axin2 (in red) is downregulated by 1,25D₃. These activities require VDR expression in tumor cells. In addition, vitamin D also acts on cells in the tumor microenvironment. We demonstrated that 1,25D₃ inhibits STAT1 activity in tumor-associated macrophages and prevents the release of IL-1β, which in a paracrine manner promotes Wnt signaling in cancer cells. 1,25D₃ can thereby regulate Wnt signaling in tumor cells that do not respond directly to 1,25D₃. VDR: Vitamin D receptor.

controls a variety of biological processes. Although 1,25D₃ has also been shown in preclinical studies to inhibit invasiveness of tumor cells and to reduce their ability to metastasize, clinical trials suggest that while vitamin D is effective in early stages of cancer, it appears to have limited activity in advanced, aggressive malignancies.

Important mechanisms whereby 1,25D₃ regulates the homeostasis of intestinal epithelium and exerts its anti-neoplastic activity is through its ability to interfere with Wnt/β-catenin signaling^[3,26,27] and to inhibit inflammation. Because inflammation can fuel Wnt signaling in colon cancer cells, the two activities may be coupled, suggesting that 1,25D₃ might exert chemopreventive activity by interrupting the link between inflammation and cancer. However, large clinical trials are required to firmly establish the preventive and therapeutic value of vitamin D in colon cancer. Such trials are complicated by the necessity of maintaining and monitoring vitamin D levels as well as clinical outcome in a large number of patients over a long period of time.

INHIBITION OF WNT SIGNALING BY VITAMIN D

The Wnt/β-catenin signaling pathway regulates the intracellular levels of β-catenin and controls the expression of β-catenin/TCF4 target genes. In normal cells, β-catenin is sequestered in a large cytoplasmic protein complex, called the β-catenin destruction box, which includes Axin and Apc and the GSK3β and CK1 kinases^[28,29]. Due to mutations in the tumor suppressor Apc, or less frequently in Axin or β-catenin, the oncogenic Wnt/β-catenin signaling pathway is abnormally activated in over 90% of colon cancers^[30].

The β-catenin destruction complex promotes β-catenin phosphorylation and its subsequent degradation. Wnt activation of its receptors, Frizzled and LRP5/6, inhibits the destruction complex and results in accumulation of β-catenin, both in the cytoplasm and in

the nucleus, where it acts as a co-activator of LEF/TCF and regulates the expression of a variety of genes. Wnt/β-catenin signaling activates genes, such as c-myc and cyclin D and thereby promotes proliferation of tumor cells. Activation of Wnt signaling also induces the expression of COX2 and survivin which increases the survival of intestinal epithelial cells. Wnt signaling has been shown to promote transcription, protein stability and to regulate nuclear localization of Snail, a transcription factor that mediates epithelial mesenchymal transition^[31,32]. In turn, Snail interacts with β-catenin and increases the expression of Wnt target genes^[33]. We showed that inflammation-induced stabilization of Snail contributes to Wnt signaling in colon cancer cells and creates a positive feedback loop initiated, and propagated, by macrophage-derived IL-1β^[34]. IL-1β was sufficient to increase the levels of Snail in colon cancer cells^[35], and the levels of both IL1β and Snail are increased in colon cancer patients (Figure 1). Importantly, Snail1 and Slug (Snail2) have been shown to inhibit the expression of VDR and to inhibit the activity of 1,25D₃^[5,36-38]. Wnt-dependent stabilization of Snail is likely to contribute to reduced expression of VDR in colon cancer patients (Figure 1).

1,25D₃ has been shown, in a VDR-dependent manner, to antagonize Wnt signaling through a variety of mechanisms. These include sequestration of β-catenin through a direct VDR/β-catenin interaction and induction of nuclear export of β-catenin. 1,25D₃ also enhances the expression of DKK1, which is an endogenous inhibitor of Wnt signaling. Furthermore, cystatin D, whose expression is strongly upregulated by 1,25D₃, inhibits Wnt signaling and the expression of its target genes, including Snail (Figure 2). Cystatin D inhibits migration and anchorage-independent growth of colon cancer cells and its silencing abrogates the anti-proliferative activity of 1,25D₃ and increases the expression of c-Myc^[39]. A comprehensive review of the mechanisms whereby vitamin D represses Wnt signaling has been published recently^[40].

Wnt activity in primary human tumors is heterogeneous, and it has been demonstrated that its activity is

regulated by factors from the tumor microenvironment. Although loss of *Apc* occurs early in adenoma development in the colon, *in vivo* progression from micro-adenomas to macroscopic tumors in *Apc*^{Min/+} mice is associated with further elevation of canonical Wnt signaling and increased expression of Wnt target genes^[41]. This suggests that enhancement of Wnt signaling beyond a threshold level sufficient for tumor initiation may be required for tumor progression and metastatic spread. Often factors from the tumor microenvironment provide signals that regulate the extent of oncogenic signaling in tumor cells. We and others have demonstrated that tumor-associated macrophages promote Wnt signaling in colon cancer cells *via* IL-1 β and TNF^[34,42]. Fibroblasts have also been shown to enhance Wnt signaling through hepatocyte growth factor^[43], confirming the role of inflammatory factors in Wnt signaling and in maintenance of cancer stem cells (see below). Leukotriene D4, which can be produced and secreted by stromal cells in the local tumor microenvironment, promotes the expression and nuclear translocation of β -catenin and thus enhances the growth of colon cancer cells^[44]. Indeed, β -catenin translocation is often detected at the invasive front of tumors^[45,46], consistent with the interpretation that stromal tissue at the invasion front provides signals to tumor cells that promote nuclear translocation of β -catenin and thus drive tumor progression. It is therefore likely that 1,25D₃ regulates Wnt signaling by targeting both the tumor microenvironment as well as the tumor cells themselves. Indeed, we have shown that vitamin D interrupts signaling between tumor cells and macrophages and thereby decreases the intensity of Wnt signaling in HCT116 colon cancer cells which are themselves unresponsive to direct effect of vitamin D^[34]. We demonstrated that this mechanism involved 1,25D₃ inhibition of STAT1 activity in macrophages, blocking the release of IL-1 and thereby restoring the sensitivity of colon cancer cells to TRAIL-induced apoptosis^[35]. This is in line with the concept that the tumor microenvironment represents an important target of chemopreventive and chemotherapeutic agents^[47].

The ability of vitamin D to regulate Wnt signaling has been confirmed in animal models. Vitamin D and its analogues reduced the number of tumors in *Apc*^{Min/+} mice^[48], associated with decreased nuclear β -catenin and reduced expression of β -catenin target genes^[49]. Likewise, dietary induction of colon tumors in mice, a model of sporadic colon cancer, accompanied by functional enrichment of Wnt signaling, is reversed by supplementation with vitamin D and Ca^[24]. *Apc*^{Min/+} mice lacking VDR have an increased number of aberrant crypt foci (ACF) and both ACFs and tumors in *Apc*^{Min/+} VDR^{-/-} mice display increased nuclear β -catenin and elevated expression of β -catenin/TCF target genes^[50]. While the number of adenomas and carcinomas was not affected by the inactivation of VDR, tumors that developed in the *Apc*^{Min/+} VDR^{-/-} mice were significantly larger, consistent with increased growth due to enhanced Wnt signaling. We recently confirmed that while targeted inactivation of VDR in intestinal cells did not alter tumor multiplicity in

Apc^{Min/+} mice, inactivation of VDR in macrophages substantially reduced *Apc*^{Min/+} tumors (submitted), confirming the important role of VDR signaling in the tumor microenvironment.

Consistent with these *in vitro* data and with studies in mice, dietary supplementation with 1,25D₃ decreased the levels of β -catenin and increased the expression of E-cadherin in normal mucosa of colon cancer patients^[51].

ANTI-INFLAMMATORY PROPERTIES OF VITAMIN D

Chronic inflammation has been shown to predispose to development of tumors, a striking example being inflammatory bowel disease, which is associated with elevated risk of colon cancer^[52]. Moreover, it appears that colon cancers that are not linked to inflammatory bowel disease are also driven by inflammation; it has been shown that regular use of NSAIDs lowers the mortality from sporadic colon cancer and inhibits adenomas in FAP patients, who inherit a mutation in the *Apc* gene^[53]. The mechanisms whereby anti-inflammatory agents inhibit progression of tumors that are not associated with overt inflammation are not fully understood. However, it has been established that cancer and several other chronic diseases are associated with para-inflammation, a low grade inflammation that is coupled to a persistent activation of the DNA damage response^[54] and the induction of DNA damage-induced soluble factors, including major pro-inflammatory cytokines, chemokines and growth factors. It is possible that anti-inflammatory agents exert their chemopreventive activity by ameliorating the pro-tumorigenic activity of para-inflammation that is associated with aging and that is observed in colon cancer patients.

Inflammatory bowel disease (IBD) is among the three most prevalent high risk conditions for colon cancer^[52]. The risk for colorectal cancer increases with the duration and the extent of the disease, consistent with a direct connection between inflammation and colon cancer development. Patients with intestinal inflammatory conditions such as ulcerative colitis (UC) and Crohn's disease (CD) have a high incidence of vitamin D insufficiency and deficiency^[55] and show reduced levels of VDR in intestinal epithelium^[56]. Likewise, higher levels of vitamin D have been shown to lower the risk of Crohn's disease^[57]. Overexpression of VDR in intestinal cells inhibits the colitis-associated increase in proinflammatory cytokines, such as TNF, IL-1 and CCL2, and protects mice from developing colitis^[56]. Finally, a vitamin D analogue has been shown to inhibit colon carcinogenesis in the azoxymethane/dextran sodium sulphate (AOM/DSS) model of ulcerative colitis^[58], suggesting that VDR signaling may avert the conversion of the inflammatory stimuli into a tumor promoting signal.

VDR knockout mice exhibit a proinflammatory phenotype associated with increased NF- κ B activity in intestine, consistent with the ability of VDR signaling to inhibit NF- κ B activation^[59]. TNF- α is a major proin-

flammatory cytokine that activates the NF- κ B signaling pathway in tumor cells and thereby regulates their growth and survival. Human colon cancers are infiltrated by inflammatory cells which secrete a variety of proinflammatory factors, including TNF- α ^[60]. Likewise, polyps arising in Apc ^{Δ 468} mice, a genetic model for intestinal cancer, showed infiltration with mast cells, and depletion of mast cells or anti-TNF- α treatment significantly suppressed polyposis in Apc ^{Δ 468} mice^[60]. Etanercept, a specific antagonist of TNF- α , also reduced the number and the size of tumors in the AOM/DSS model, confirming a role of TNF- α in inflammation-promoted intestinal tumorigenesis. More intriguing was the observation that inhibition of TNF- α blocks the accumulation of β -catenin mutations in intestinal cells, suggesting a mutagenic role of TNF- α ^[61]. Pharmacological inhibition of TNF- α by neutralizing TNF- α antibodies is very effective in alleviating inflammation in IBD patients^[62] and inhibitors of TNF- α have also been tested as potential agents for the treatment of colon cancer. Unfortunately, TNF- α inhibitors have been linked to a broad range of infections and to the development of lymphomas and skin and lung cancer, limiting their clinical utility.

An alternative approach to targeting TNF/NF- κ B-mediated inflammation and interrupting the link between inflammation and cancer may be offered by vitamin D. 1,25D₃ inhibits the interaction of peripheral blood mononuclear cells and colon cancer cells and inhibits the production of TNF^[63] and blocks NF- κ B signaling, a major TNF signaling pathway. VDR physically interacts with IKK β ^[59] and vitamin D downregulates the expression of NF κ B target genes, such as Puma^[56], which play a major role in the survival of cancer cells. In addition, 1,25D₃ has been shown to downregulate the expression of Toll-like receptors 2 and 4 (TLR2 and TLR4) on human monocytes, resulting in hyporesponsiveness to TLR activating ligands^[64,65]. Inhibition of TLR signaling by vitamin D₃ has been suggested to reduce AOM/DSS-induced colon cancer^[66], pointing to a convergence of the chemopreventive and anti-inflammatory properties of vitamin D₃.

NF- κ B is not the only oncogenic signaling pathway activated in tumor cells by inflammatory factors. We have shown that TNF enhances Wnt signaling in β -catenin mutant colon cancer cells^[34], and established that macrophage-derived factors activate Wnt signaling in colon cancer cells through NF- κ B signaling^[42]. Oguma *et al*^[67] demonstrated that TNF- β promotes Wnt signaling also in gastric cancer cells, which was independent of NF- κ B in this tissue.

The HCT116 colon cancer cells have a functional VDR, but do not respond to 1,25D₃ treatment with growth arrest, apoptosis or differentiation. However, we demonstrated that in the presence of macrophages, 1,25D₃ reduced Wnt signaling in these seemingly vitamin D unresponsive cells by interrupting signaling between tumor cells and macrophages. 1,25D₃ inhibits STAT1 activity and prevents tumor cell-induced release of IL1 from macrophages and thereby prevents inflammation-induced Wnt signaling in colon cancer cells^[34] (Figure 2). Accordingly, 1,25D₃ inhibits the ability of macrophages to increase proliferation

and survival of colon cancer cells. Among genes that were repressed by 1,25D₃ in tumor cells in a macrophage-dependent manner were cyclin D1 and c-myc, consistent with the finding that 1,25D₃ prevented macrophage-induced clonogenic growth of HCT116 cells. Therefore, 1,25D₃ can exert its tumor-preventive activity by normalizing the tumor microenvironment, and it can inhibit inflammation through a variety of mechanisms.

Diet-induced obesity, a risk factor for colon cancer, is also associated with increased expression of TNF- β in the intestine. In this settings, TNF- β has also been shown to be coupled to inactivation of GSK3- β and increased expression of β -catenin and c-myc, suggesting that obesity increases the risk of colorectal cancer by promoting inflammation^[68]. Indeed, western style diet (WSD), sufficient to initiate intestinal tumorigenesis in mice^[24], has been shown to trigger an inflammatory response in mice, accompanied by the accumulation of macrophages in intestinal mucosa and increased levels of circulating proinflammatory cytokines, including IL-1 β , CCL5 and CCL2^[69]. Importantly, dietary supplementation with vitamin D and Ca prevents WSD-induced increases in inflammatory markers and inhibits intestinal tumorigenesis^[24,69]. Dietary supplementation with 1,25D₃ reduced markers of inflammation, including C-reactive protein (CRP), TNF, IL-1 β , IL-6 and IL-8 also in colon cancer patients^[70], strongly suggesting that 1,25D₃ protects from colon cancer, at least in part, by decreasing inflammation.

CONCLUSION

Calcitriol, the most active form of vitamin D₃, acts as a potent steroid hormone that binds to VDR and thereby alters the expression of a variety of genes that regulate growth, differentiation and survival of epithelial cells. Epidemiological studies suggest that deficiency of vitamin D increases the incidence of colon cancer and also has a negative impact on the survival of colon cancer patients. The ability of 1,25D₃ to interfere with Wnt signaling and to ameliorate inflammation is likely to contribute to its anticancer activity. The optimal form and adequate concentration of vitamin D that have cancer preventive activity should be established, and randomized clinical trials are needed to confirm that 1,25D₃ alone, or in combination with other cytotoxic agents, offers therapeutic benefits.

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WJGO covers topics concerning carcinogenesis, tumorigenesis, metastasis, diagnosis, prevention, prognosis, clinical manifestations, nutritional support, molecular mechanisms, and therapy of benign and malignant tumors of the digestive tract. The current columns of WJGO include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography. Priority publication will be given to articles concerning diagnosis and treatment of gastrointestinal oncology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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Statistical data

Write as mean \pm SD or mean \pm SE.

Statistical expression

Express t test as t (in italics), F test as F (in italics), chi square test as χ^2 (in Greek), related coefficient as r (in italics), degree of freedom as ν (in Greek), sample number as n (in italics), and probability as P (in italics).

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Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μ g/L; CO₂ volume fraction, 50 mL/L CO₂, not 5% CO₂; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

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Italics

Quantities: t time or temperature, c concentration, A area, l length, m mass, V volume.

Genotypes: *gyrA*, *arg 1*, *c myc*, *c fos*, *etc.*

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Biology: *H. pylori*, *E coli*, *etc.*

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