Anti-*Helicobacter pylori* antibody responses specific for VacA do not trigger primary biliary cirrhosis-specific antimitochondrial antibodies

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Recently, Goo *et al.* [1] reported the induction of primary biliary cirrhosis (PBC)-like pathology in a C57BL/6 mouse infected with *Helicobacter pylori* and speculated that anti-VacA antibodies can induce experimental disease and antibodies against the dominant pyruvate dehydrogenase complex E2 subunit (PDC-E2) autoantigen, through a mechanism of molecular mimicry.

We have investigated in detail the pathogenic role of molecular mimicry involving microbial agents such as *H. pylori* and PDC-E2 autoepitopes [2–5] and we would like to raise a few points:

We have noted that Goo *et al.* [1] have not tested whether anti-PDC-E2 antibodies are present in the mouse with PBC-like pathology.

We have tested 70 patients with PBC (50 anti-PDC-E2 positive and 20 anti-PDC-E2 negative) [3,4] and 100 demographically matched controls (70 with chronic hepatitis C and 30 normal), all from Greece, for reactivity to VacA of *H. pylori* by immunoblotting (Euroimmun, Lübeck, Germany). The presence and levels of IgG class anti-VacA antibodies did not differ between anti-PDC-E2 positive and PDC-E2 negative PBC cases (17 of 50, 34% vs. 6 of 20, 30%) or between PBC patients and controls (33 vs. 31%).

Solid-phase inhibition experiments in anti-VacA/PDC-E2 double reactive cases while abolishing reactivity to PDC-E2, left unaffected reactivity to VacA *H. pylori*. The reciprocal experiment using VacA as inhibitor left unchanged anti-PDC-E2 antibody reactivity.

Through a BLAST2p protein–protein database search, we have found insignificant similarities between VacA of *H. pylori* and human PDC-E2 (30–61% homology for the best five matches). None of the VacA/PDC-E2 mimics involved the – critical for antibody binding – PDC-$E2_{212–226}$ core epitopic region.

Our data suggest that anti-VacA antibody responses do not cross-react and are not associated with PBC-specific anti-PDC-E2 responses. Anti-VacA *H. pylori* antibodies are most likely irrelevant to the pathogenesis of PBC.

Acknowledgement
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Signature genes for both hepatoblastoma and hepatocellular carcinoma

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A major goal in current hepatocellular carcinoma (HCC) research is to define molecular or gene signatures that govern initiation, maintenance and progression of the malignant tumours. The gene signature should be helpful in classifying tumour stages and predicting prognostic outcomes, such as metastasis, patient survival rate and recurrence of the tumours after resection. It is also highly desirable to use the gene signature to design targeted therapies or the so-called personalized medicine.

Most recently, Cairo and colleagues [1] reported signature genes for hepatoblastoma (HB), an infant liver tumour that is clinicopathologically distinctive

Fig. 1

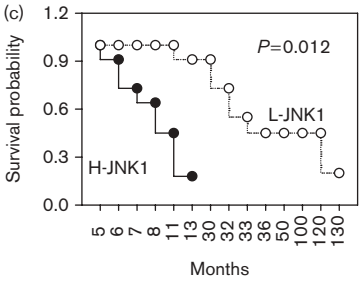
(a)

Up in H-JNK1/L-JNK1		Up in HB*	Down in H-JNK1/L-JNK1		Down in HB*
Genes	fold		Genes	fold	
S100P	611		GYS2	−122	0.1
S100A8	371	a	F9	−141	0.1
AGR2	309		CFHR5	−148	
S100A9	278	a	NAT2	−151	0.2
CCL20	266		ADH1B	−151	0.1
PEG10	206	14.9	SPP2	−154	a
CD24	163	6.7	LECT2	−155	
NTS	143		SLC22A1	−156	0.1
SPP1	126		SLC27A5	−156	0.2
PLAC8	121	a	BAAT	−173	
H19	92		GLYATL1	−175	
SPINK1	78		FMO3	−183	0.1
GALNT7	63		RTP3	−217	
HMGA2	61	5.5	ACSM2B	−222	
C1orf106	61		CYP8B1	−224	
AMIGO2	55		AFM	−239	a
AFP	54	35	CYP2C9	−242	0.2
XIST	52		CYP2E1	−244	0.2
UBE2C	51		PCK1	−251	0.1
NEBL	49		OTC	−276	
SOX4	48	3.0	HSD17B6	−298	0.1
IER3	45		GLYAT	−458	0.2
EGLN3	42		CYP2C8	−525	0.0
TMED3	40		ADH4	−535	
PRKAR2B	40		AQP9	−684	0.2
IGFBP3	39		SERPINC1	−711	a
S100A4	39		SLC10A1	−975	0.1
BASP1	38	b	HSD11B1	−1040	0.1
CXCL13	37		CYP3A4	−1458	
KRT19	34	10.5	HPD	−1864	0.2

(b)

16 HB Signature genes	Fold of H-JNK1/L-JNK1
GHR	−19.49
APCS	−9.44
ALDH2	−9.12
C1S	−41.83
AQP9	−684.5
CYP2E1	−243.6
APOC4	−109.7
HPD	−1864
NLE	1.1
RPL10A	1.92
E2F5	1.66
BUB1	6.7
DLG7	13.2
IGSF1	1.34
AFP	53.6
DUSP9	7.7

(c)



Overlapping of signature genes between higher JNK1 hepatocellular carcinoma (HCC) and hepatoblastoma (HB). (a) The data of gene profiling by Chang *et al.* [2] were reanalyzed by removing the probes with 'absent' call and FDR correction. Ten out of the 30 most upregulated genes and 20 out of the 30 most downregulated genes in the higher JNK1 HCC are represented by the upregulated and downregulated genes in the HB or rC2 HB, respectively. H-JNK1: higher JNK1 HCC; L-JNK1: lower JNK1 HCC. a: up or down in later stage HB; b: up or down in early stage HB; (*): ratio of rC2/rC1. (b) The expression levels of the 16 signature genes proposed by Cairo *et al.* [1] in the H-JNK1 HCC versus L-JNK1 HCC. (c) Kaplan–Meier plot of the survival probability for 22 male patients with H-JNK1 HCC ($n=11$) and L-JNK1 HCC ($n=11$). $P=0.012$.

from HCC. By comparing their data with our recent report on JNK1 activation and HCC gene profiling [2], a striking similarity between the genes in robust Cluster 2 (rC2) HB and the genes associated with the JNK1 activation status was noted. For the top 100 increased and bottom 100 decreased expressed genes in rC2 HB (Supplemantary Table S7 of Cairo *et al.*), 92 and 94% of these genes are presented among the most increased and decreased expressed genes in the HCC with higher JNK1 activation, respectively. Vice versa, 10 out of the 30 most upregulated genes and 20 out of the 30 most downregulated genes in the HCC with higher JNK1 activation are represented by the upregulated and downregulated genes in the HB or rC2 HB, respectively (Fig. 1a). A significant agreement of the 16 signature genes differentiating rC2 and rC1 HB (Fig. 6a of Cairo *et al.*) and the signature genes for the HCC with higher JNK1 activation was evident. The listed upregulated and downregulated genes in rC2 HB are fully overlapping with the upregulated and downregulated genes in HCC with higher JNK1 activity (Fig. 1b). In addition, as observed in rC2 HB, the liver progenitor markers AFP, TACSTD1, KRT19, KRT7 and imprinted genes are also substantially upregulated in the HCC with higher JNK1. Evidence to further support the similarity between rC2 HB and the HCC with higher JNK1 activation is the patient survival probability. The overall survival of both rC2 HB patients and HCC patients with higher JNK1 was substantially compromised (Fig. 1c).

There are extensive debates on whether HCC arises exclusively from dedifferentiation of mature hepatocytes or maturation arrest of liver stem cells [3]. As HB is a stem-like cell carcinoma, the similar gene signature in HB and HCC, thus, strengthens the notion that many HCCs are developed from liver stem cells and that the HB might be an early stage of HCC. In addition to HB and HCC, some of the liver progenitor markers are also overexpressed in intrahepatic cholangiocarcinoma [4].

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Will skepticism stop the evolution of endoscopic GERD treatment?

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Gastroesophageal reflux disease (GERD) has a prevalence of 10–20% in Western Europe and North America and was defined in the Montreal consensus as a condition that develops when the reflux of stomach contents causes troublesome symptoms and/or complications [1]. Characteristic symptoms are retrosternal burning (heartburn) and regurgitation. GERD can cause esophageal complications associated with mucosal injury that include esophagitis, stricture, Barrett's esophagus and esophageal cancer, and also extra-esophageal complications including laryngitis, cough, asthma, and dental erosions. Therapeutic options for GERD have been lifestyle changes, antisecretory medication and surgery, but recently several types of endoscopic therapies have also been developed.

To date, the medical management of GERD with proton pump inhibitor (PPI) therapy has been highly effective in controlling gastric acid secretion. Among GERD patients, 70–80% are asymptomatic on PPIs during their whole life or at least for a long period of time [2]. However, the disease is not cured and therefore many patients require a lifelong commitment to PPIs. Grounds for searching alternatives are also based on the high medication costs and potential side-effects. Emerging evidence indicates that long-term PPI treatment is associated with an increased risk of osteoporosis, enteric infections, and pneumonia [3–5]. The alternative to drugs has been antireflux surgery, and the laparoscopic Nissen fundoplication has become the gold standard. Although very effective in creating an antireflux barrier, it remains an invasive procedure with side-effects, such as dysphagia, gas bloating, and excessive flatulence, which can highly impact on a patient's quality of life [6].

In view of the downsides of current treatments, a minimally invasive endoscopic treatment that obviates the need for PPIs is in demand. Several endoscopic techniques have been developed based on various mechanisms of action: thermal tissue remodelling by radiofrequency delivery [7], injection of bulking agents [8], creation of esophageal tissue pleats by mucosal suturing [9], and full thickness fundoplication [10,11]. The first three techniques aimed for inhibition of the retrograde flow of gastric contents by narrowing the esophagogastric junction. Although early results have been promising, sham-controlled trials showed disappointing outcomes and most first-generation devices have been withdrawn from the market. The success of the surgical repair was, however, not based on narrowing the esophagogastric junction, in contrast it involves the creation of a 'very loose wrap' to restore the gastroesophageal valve [12]. Therefore, endoscopic full thickness fundoplication techniques, designed to mimic the surgical anterior fundoplication hold more promise, especially as the surgical anterior repair was shown effective in long-term reflux control with less side-effects compared with Nissen's 360 degrees fundoplication [13].

The Plicator system (NDO Surgical Inc., Mansfield, Massachusetts, USA) enabled the first full thickness endoscopic fundoplication performed in GERD patients. Outcomes were not as good as after surgery, but sham-controlled trials did show not only improved symptom scores, but also a reduction in lower esophageal acid exposure [10]. The recently introduced transoral incisionless fundoplication (TIF), using the EsophyX device (EndoGastric Solutions Inc., Redmond, Washington, USA) is thought to resemble the surgical repair to an even greater extent. By the deployment of multiple polypropylene fasteners, TIF enables the creation of an endoscopic anterior full thickness fundoplication that restores the angle of His and creates an individually tailored gastroesophageal valve. An early study on GERD patients showed effectiveness in subjective and objective outcome measures after 1-year follow-up [11]. Still, not all patients were cured and TIF has not yet been subjected to the test of a randomized (sham) controlled trial.

However, conducting such a trial has become a challenge. In this edition of the journal, Eckardt *et al.* [14] report the results of their study in which the patient recruitment process for a phase 2 cohort study using TIF was analyzed. They hypothesized that the GERD market for a new endoscopic antireflux procedure may be overestimated. Their recruitment sources were 50 referring private practices, 23 hospitals, and three advertise ments in major newspapers. The assessment of the