The complexity of cancer origins at the gastro-oesophageal junction

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ABSTRACT

Chronic acid-biliary reflux and *Helicobacter pylori* infection are instrumental environmental drivers of cancer initiation and progression in the upper gastrointestinal tract. Remarkably, although these environmental carcinogens are quite dissimilar, the tumour progression cascade these carcinogens engender is highly comparable. For this reason, studies of malignant progression occurring at the anatomic borderland between the oesophagus and the stomach have traditionally lumped junctional adenocarcinomas with either oesophageal adenocarcinoma or gastric adenocarcinoma. Whilst studies have revealed remarkable epidemiological and genetic similarities of these cancers and their associated premalignant conditions, these works have also revealed some key differences. This highlights that further scientific effort demands a dedicated focus on the understanding of the cell-cell interaction between the epithelium and the local microenvironment in this anatomic region. We here review available evidence with regards to tumour progression occurring at the gastro-oesophageal junction and contrast it with available data on cancer evolution in the metaplastic oesophagus and distal stomach.

KEY WORDS

Gastric cancer, oesophageal adenocarcinoma, Barrett oesophagus, gastro-oesophageal junction, intestinal metaplasia, *H. pylori*, cardia cancer.

DEFINITION AND BACKGROUND

Adenocarcinomas of the gastro-oesophageal junction (GOJ) represent a heterogeneous tumour entity. The *International Gastric Cancer Association* (IGCA) and the *International Society of Diseases of the Esophagus* (ISDE) broadly define all tumours positioned 5cm above and below the proximal ends of the gastric folds as GOJ cancer. The most widely used clinical classification for these cancers has been proposed by Siewert and colleagues and is based on the epicentre of the main tumour mass in relation to the GOJ[1]. Type I tumours are positioned 1-5cm above the GOJ. Type II cancers are the classic cardia cancers, with the main bulk centred around the GOJ (1cm above and up to 2cm below the GOJ)[2,3]. Type III GOJ cancers are located 2-5cm below the junction and represent mainly proximal gastric cancers.

Despite attempts to establish broad acceptance of a uniform classification, comparison of epidemiological data is hampered by the continued use of a mixture of different definitions and terms. GOJ cancers have been included in studies on either oesophageal or gastric cancer, but also been summarised under terms such as 'Barrett cancer' or 'cardia cancer'. A Swedish register study analysed data on oesophageal and cardia cancers in the late 1990s. The second half of the 20th century brought a marked increase in the incidence of GOJ cancers, especially in industrialised countries[4]. This was accompanied by a decline of both distal gastric cancer and oesophageal squamous cell carcinoma, with GOJ tumours overtaking the former entities in absolute numbers[5,6]. There are considerable regional differences in the respective incidence rates of these cancers[7,8]. In Europe, the increasing incidence of oesophageal adenocarcinoma is most clearly seen in Northern countries[8–11]. In contrast to the developments in Europe and North America, the incidence of GOJ cancers in Asia has remained comparably low, with non-junctional gastric cancers still predominating. Nevertheless, with the adoption of a Western life style and diet, GOJ cancers are now increasing in China as well. In the following we will shed light on factors driving cancer initiation and progression at the GOJ.

RISK FACTORS

Several key risk factors for GOJ cancers have been described (**Figure 1**). The effect size of some of these risk factors differs when compared to oesophageal or gastric adenocarcinoma.

Gastro-oesophageal Reflux and Barrett oesophagus

General risk factors for GORD (e.g. obesity, presence of a hiatus hernia) and the duration of reflux symptoms are all associated with an increased risk of oesophageal adenocarcinoma[12]. Patients with

recurrent severe retrosternal burning and regurgitation have a nearly 8-fold increased risk of oesophageal adenocarcinoma and 2-fold increased risk of cardia cancer[13]. The risk increases with more frequent, more severe, and longer-lasting symptoms, with an odds ratio (OR) for oesophageal adenocarcinoma of 43.5 (95% CI: 18.3-103.5) in specific risk groups. *Vice versa*, patients with oesophageal adenocarcinoma present reflux symptoms in only 61% of cases, meaning just over half of all oesophageal adenocarcinoma patients have a history of reflux complaints[14]. Likewise, only 38% of patients with GOJ cancers report a history of reflux complaints[14]. Clearly, the interaction between reflux, reflux perception, and cancer risk is multifactorial and remains an important avenue for clinical research.

Barrett oesophagus is a complication of chronic gastro-oesophageal reflux and is an established precursor lesion of oesophageal adenocarcinoma. It is defined as the metaplastic replacement of the squamous lining of the distal oesophagus by columnar glandular mucosa, extending directly from and above the GOJ[15]. This metaplasia can show gastric, intestinal, or pancreatic differentiation. A diagnosis of Barrett oesophagus can be made if the typical endoscopic finding of tongues or islands of metaplastic lining is seen above the top end of the gastric folds and then confirmed on biopsy[15–17].

The cancer risk attributed to Barrett oesophagus remains unclear, in part due to underdiagnosis of Barrett oesophagus in the general population as well as differences in disease coding between health care systems. A meta-analysis on data from 1966 to 1998 put the risk of malignant progression at 0.5% (95%CI 0-3%) per patient per year[18]. It is likely that the real-world risk is even lower. The risk of progression towards dysplasia correlates with the length of the metaplastic segment[16,17,19–21], although it remains to be demonstrated if this is due to the length of the BE segment *per se* or co-determined by other factors such as local active inflammation or genetic constitution. Importantly, although intestinal metaplasia (IM) is traditionally seen as a *sine qua non* for the diagnosis, evidence has been building that IM is not an obligate precursor lesion for cancer progression. An observational study from the UK followed 712 patients with either specialised IM (SIM) at the cardia or non-intestinal glandular mucosa in the distal oesophagus for 12 years[22]. The incidence of oesophageal adenocarcinoma was 0.34% per year, with no statistically significant difference between patients with or without intestinal metaplasia. Takubo examined 141 early cancers of the distal oesophagus and found that the adjacent non-dysplastic epithelium revealed cardiac or fundic (oxyntic) type mucosa rather than intestinal type lining in the majority of patients[23]. In more than 50% of these cases, the endoscopic resection specimen did not contain any IM at all. Although this study suggested that these cancers had derived from gastric-type epithelium, direct evidence of gastric lineage derivation requires lineage tracing in patient material. Lavery *et al.* traced the origin of a single oesophageal adenocarcinoma to an expanding epithelial clone of cardia-type epithelium[24]. This precursor clone

demonstrated a pathogenic P53 mutation which had undergone loss of heterozygosity in the resulting adenocarcinoma, formally demonstrating that non-dysplastic cardia epithelium can clonally expand and carry oncogenic precursor mutations. Previous data to this point had been raised in genomic analyses of BE tissues from patients with or without later progression to HGD or EAC, showing higher numbers of *TP53* mutations in BE from patients with subsequent progression. These mutations were frequently detected before the onset of dysplasia or appearance of substantial changes in genomic copy number[25,26]. It remains to be determined which cellular components and factors within the local mucosal microenvironment contribute to these processes.

Helicobacter pylori

The micro-aerophile, spiral bacteria *Helicobacter pylori* (*H. pylori*) has been established as the main risk factor for non-junctional gastric cancer and is classified as a definite (group 1) carcinogen by the WHO[27,28]. Several meta-analyses attribute a three-fold increase risk for gastric cancer to patients infected with *H. pylori*[29–32]. The calculated risk is higher when presence of bacterial virulence factors is taken into account. The impact of expression of the *cytotoxic gene A* (*cagA*) that encodes a type IV secretion system injecting *H. pylori's* key pathogen into the host's epithelial cells is most studied in this respect [33,34].

The role of *H. pylori* infection in the pathogenesis of cancers at the GOJ is less clear and remains heavily debated, but recent evidence indeed suggests a role for *H. pylori* in the development of cardia cancer, especially in case of CagA-positive infection[35]. However, this effect was less pronounced across studies when compared with non-cardia or non-junctional gastric cancer. It has therefore been suggested that other high-risk features need to be present to facilitate junctional carcinogenesis, such as a high regional incidence of gastric cancer and certain histopathological characteristics[36]. Another influencing factor is the regional prevalence of GORD and Barrett oesophagus. Data from 30,000 individuals from the Chinese province of Linxian showed a similar risk of cardia and non-cardia gastric cancers for patients infected with *H. pylori*. The reason appears to be the negligible prevalence of Barrett oesophagus in the region, which mitigates the impact of GORD on GOJ cancer development[37]. This effect can be simulated when Barrett-associated cancers and GOJ type I tumours are excluded from risk association analyses. The remaining patients (GOJ type II and III) show a prevalence of *H. pylori* comparable to that seen in patients with distal gastric cancer[38–40].

The interaction of *H. pylori*-induced chronic gastric inflammation and reflux-related effects at the GOJ is highly complex. Epidemiological evidence suggests an inverse relationship between the prevalence of *H. pylori* and incidence of oesophageal adenocarcinoma. A recent meta-analysis of 72 studies including 84,717 cases and 390,749 controls confirms this inverse association with a lower OR

for oesophageal cancers in *H. pylori*-positive subjects (OR 0.68, 95% CI: 0.58-0.79)[41]. In contrast, a retrospective study on 36,803 US veterans could not confirm a statistically significant association between *H. pylori* status - or *H. pylori* treatment status - and incidence of oesophageal adenocarcinoma[42]. A Swedish nationwide population-based cohort study (n=81,919) confirmed a significant association of *H. pylori* status (based on eradication history) with presence of Barrett oesophagus resulting in a standardised incidence ratio (SIR) of 3.67 (95% CI: 3.15-4.25)[43]. Interestingly, this effect decreased with longer follow-up time after eradication, and there was no statistically significant association with oesophageal cancer in this study.

Other studies focussed on the link between *H. pylori* infection and non-cancerous reflux conditions. A meta-analysis of 17 studies (n=6,889) documented an increased risk of erosive reflux oesophagitis after eradication (OR 1.67, 95% CI: 1.12-2.48), but not of GORD-related symptoms (OR 1.04, 95% CI: 0.84-1.29)[44]. The *Barrett and Esophageal Adenocarcinoma Consortium* (BEACON) enrolled 1,308 Barrett patients, 1,388 population-based controls, and 1,775 non-Barrett GORD controls in their study with a sub-analysis also showing a reduced risk of Barrett oesophagus in *H. pylori*-positive patients (OR 0.44, 95% CI: 0.36-0.55)[45]. It is of note that this effect was only seen when cases were compared to population-based controls, but this effect was lost when cases were compared to GORD controls (OR 0.96, 95% CI: 0.67-0.137). A Japanese study on 41,065 asymptomatic individuals undergoing a medical survey showed an inverse association of *H. pylori* infection for long segment Barrett oesophagus (>3cm; OR 0.42, 95% CI: 0.16-0.91), but confirmed the infection as risk factor for shorter Barrett segments (OR: 1.66, 95% CI: 1.56-1.78). This effect was only present in absence of reflux oesophagitis, but did not persist in presence of reflux-induced inflammation (OR 1.07, 95% CI: 0.84-1.37)[46]. Together, these studies highlight the need for a more comprehensive understanding of the pathophysiological link between *H. pylori* and reflux-provoked pathology. There are strong indicators suggesting that this interplay is orchestrated by the local microenvironment. One factor might be the shift in the microbial community of the stomach after eradication of *H. pylori*[47–49].

Epstein Barr Virus (EBV) infection has been established as a second infectious risk factor for gastric cancer. EBV-associated carcinogenesis is outside the focus of this review, except for a brief comment here. A recent meta-analysis of data from 20,361 patients confirmed a pooled EBV prevalence in 8.7% of gastric cancer patients (95% CI: 7.7-9.9)[50]. The associated OR for gastric cancer was significantly higher in studies in which matched pairs of non-cancerous mucosa were included (OR 18.56, 95% CI: 15.68-21.97) compared to studies with non-matched pairs design (OR 3.31, 95% CI: 0.95-11.54). Prevalence was highest for junctional cancers (12.47%), slightly lower in the gastric body

(11.68%) and lowest in the gastric antrum (6.29%)[50]. This geographical difference with regards to EBV-associated gastric carcinogenesis remains unexplained at present.

Other risk factors

Obesity is a confirmed risk factor for GORD and hence also relevant in the pathogenesis of GOJ cancers. A meta-analysis from 2007 on two cohort and twelve case-control studies reported a 2-fold risk increase for oesophageal adenocarcinoma in either males or females with a BMI >25kg/m^2[51], with further risk increases with higher BMI. Studies were more heterogeneous regarding the effect on junctional cancer and confirmed an effect only when studies from the US and Europe were included (OR 1.5). An increased risk for both oesophageal and junctional cancer has been described in obese patients at young ages, i.e. less than 30 years of age[52]. More recent studies indicate that BMI is not an optimal parameter to investigate the association between obesity and cancer risk. Waist circumference or waist-to-hip ratio had already been suggested in the past as a more precise surrogate indicator[53–56]. A statistically significant association of obesity with non-cardia gastric cancer could not be confirmed in most studies[56–58]. In addition to the indirect effect of obesity on the GOJ cancer risk by a higher likelihood of reflux, a direct carcinogenic influence has been hypothesized due to micro-metabolic effects of visceral adipocytes.

Most studies on the risk of gastro-oesophageal cancer and tobacco smoking confirm a dose-dependent risk increase linked to both the duration of smoking and the number of pack-years [59,60]. A meta-analysis from 2011 comprising 33 studies showed a significant risk increase (RR 1.62-2.32) for smokers to develop cancer of the oesophagus and the cardia[61], with similar data being available for non-junctional gastric cancer[62,63]. A more recent assessment of data from 23 studies suggested a significant publication bias of previous meta-analyses resulting in an overestimation of the attributable risk by 22% for cardia cancer[64].

Although the negative impact of heavy drinking has been well-described, an association of alcohol with the development of cardia cancer has not been established thus far[62,65–68].

Studies suggest that a Western diet increases the risk for oesophageal adenocarcinoma and cardia cancer. The carcinogenic effect of a high intake of red and processed meat has been confirmed in numerous studies, but the strength of the association can vary depending on tumour location[69–72]. While caffeinated drinks have been linked to an increased risk of cardia cancer, a sugar (sucrose) rich diet, sweetened desserts and beverages have been associated with a risk increase for oesophageal adenocarcinoma only[73–75].

A general positive effect on gastro-oesophageal cancer risk is attributed to a Mediterranean diet[69,76]. A high proportion of fruit and vegetables reduces the risk for cancer at the GOJ by nearly

half[77,78]. Various factors are discussed as drivers of this protective effect including vitamins A, C, E and flavonols, possibly due to a reduction in free radicals and a modulation of a local inflammatory response[79–82].

ANATOMIC ORIGIN

As mentioned above, the traditional classification of GOJ tumours as either oesophageal or gastric cancers is mainly driven by the need to facilitate (usually surgical) treatment strategies and ignores aetiological or molecular background of the disease. While the Siewert classification and the UICC TNM system classify tumours strictly according to the location of the main tumour mass[1,83], other suggestions have incorporated clinical and paraclinical surrogate parameters, such as serum markers, histopathological changes of the surrounding mucosa, and clinical symptoms (GORD). Kenneth McColl's group in Glasgow analysed the association of cardia cancers with the presence of premalignant conditions of the gastric mucosa, *H. pylori* status, and clinical history of reflux symptoms[40,84]. As expected, distal gastric cancers showed a strong association with *H. pylori* infection and presence of gastric atrophy, whereas patients with oesophageal adenocarcinoma did not show this link, but instead revealed a higher prevalence of reflux symptoms[40,84]. Reflux-associated cancers were seven times more often of the intestinal than the diffuse type, whereas this distribution was equal for the gastric phenotype. In this study, cardia cancers were stratified by oesophageal or gastric phenotype. The association of a group of cardia cancers with glandular atrophy of the stomach has been established numerous times. For example, a European multicentric case-control study on 360,000 individuals reported an OR of 11.0 (95% CI: 3.0-49.0) for cardia cancer in patients with severe chronic atrophic gastritis[35]. This was confirmed in a meta-analysis[85].

While the main driver of IM and atrophy in the stomach is thought to be *H. pylori* infection, reflux is the key risk factor at the GOJ and above. Patients with IM-positive Barrett oesophagus show more erosive oesophagitis and reflux symptoms, and presence of IM adjacent to GOJ type I and II tumours is also associated with a history of reflux symptoms[86]. A recent study showed a positive correlation of IM at the GOJ with increased acid exposure in the distal oesophagus, measured by 24 hours pH-impedance monitoring, as well as with the frequency of acid reflux episodes[87]. There was no association of IM with *H. pylori* infection in this study. On the other hand, patients with IM at the cardia without reflux features are usually *H. pylori*-positive and display further metaplastic foci within the stomach[88,89]. This is paralleled by molecular studies showing that the expression of the intestinal transcription factor CDX2 correlates with the degree of local inflammation in patients with *H. pylori* infection, but not in those with reflux disease[90]. This suggests different molecular pathways

which can trigger metaplastic transformation and a key role of the local microenvironment in different parts of the upper GI tract. It does not, however, answer the open question about the further fate of the metaplastic glands, i.e. if these are real precursor lesions or rather bystanders of carcinogenesis. Recent findings have further corroborated that intestinal metaplasia is not an obligate precursor and indeed raise the interesting hypothesis that goblet cell differentiation and lower levels of Notch signalling in the setting of columnar-lined oesophagus might be associated with a *reduced* risk of cancer progression[91]. The multi-centre consortium from the National Cancer Institute's *Barrett Esophagus Translational Research Network* (BETRNet) performed a multicentre cross-sectional study in 164 patients with Barrett oesophagus, with and without dysplasia or early oesophageal adenocarcinoma, and demonstrated that increased Notch signalling in Barrett oesophagus decreases goblet cell differentiation and promotes progression to oesophageal adenocarcinoma.

HISTOPATHOLOGY

Data outlined above suggest solid epidemiological evidence for a defined risk profile of junctional adenocarcinoma characterised by a combination of H. pylori infection and chronic reflux. Why then should it be so hard to disentangle the histogenetic origins of glandular carcinomas at the gastro-oesophageal junction? The crux of this debate is that we still do not have a proper understanding of the normal histo-anatomy at the GOJ. This requires an overview of the various glandular phenotypes that are found in this region with a special focus on epithelial metaplasia (**Figure 2**).

Intestinal metaplasia is traditionally divided into the complete and the incomplete subtype[25,92,93]. The former is found more commonly in the gastric body and the cardia, whilst the latter is more common in the gastric antrum as well as in Barrett oesophagus. This regional distribution is likely to be of significance but the underlying mechanism is completely unclear. Note that in Barrett oesophagus incomplete intestinal metaplasia is also called 'specialised metaplasia'. This epithelium displays a rich admixture of goblet cells against a background of columnar cells resembling gastric foveolar cells. These contain acid sialomucin and neutral mucin of normal gastric foveolar cells. Indeed, mucin core proteins commonly used to categorize lesions of gastric or intestinal type are both found in specialised epithelium. Thus, both MUC2 and MUC3 normally seen in intestinal epithelium, and MUC1, MUC5AC and MUC6, characteristic of gastric epithelium, are found in incomplete intestinal metaplasia, a pattern also reflected in trefoil peptide expression. There is also a topographic expression pattern of these mucin core proteins along the gland axis with MUC5AC/TFF1 and MUC2/TFF3 found in the upper portion of the gland, and MUC6 and TFF2 localised in the mucous cells

of the Barrett's gland base. This compartmentalisation strongly resembles the basic architecture of the pyloric gland in the gastric antrum.

Leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5) is thought to locate intestinal stem cells in mouse and man and in situ hybridization shows LGR5 mRNA expression at the junction of the MUC5AC+/TFF1+ cells and the MUC6+/TFF2+ cells indicating the site of the stem cell niche[25]. From this position in the glands a bidirectional cell flux can be observed reflecting their kinetic organisation, with maximum proliferative activity in the mid-portion, as shown by Ki67 immunolabelling. Complete type intestinal metaplasia reveals an epithelial lining replete with enterocytes and goblet cells secreting sialomucins and containing MUC2 intestinal mucin, but without gastric mucin core proteins. Paneth cells are localised towards the base of the gland.

In contrast to complete intestinal metaplasia – which phenocopies small intestinal glandular epithelium in every respect – incomplete intestinal metaplasia is a phenotype almost literally halfway between gastric and small intestinal, which is not normally found anywhere in the tubular gut. In addition to these metaplastic lineages, the microscopic phenotype of the cardia can include a wide variety of cell lineages and proliferative units. The basic glandular unit of the cardia is the mucinous gland without parietal cells, variously called cardiac epithelium by some and non-goblet columnar epithelium by others. In these glands, the superficial glandular epithelium is composed of foveolar mucinous columnar cells without goblet cells. The base is formed of acini lined by MUC6+/TFF2+ mucinous cells, much like the specialised gland base discussed above. Secondly, oxynto-cardiac glands are found and are formally defined as glands containing a mixture of mucous cells and parietal cells. These glands phenocopy normal pyloric epithelium, save for the absense of gastrin-producing G cells which are unique to the native antrum. This gland phenotype also displays a basic ground plan of superficial foveolar glandular epithelium and a mucous base formed of acini lined by MUC6+/TFF2+ mucinous cells. Contrary to common belief, parietal cells are *not* restricted to corpus-type mucosa and are also abundantly found in normal pyloric mucosa and cardiac mucosa.

A key issue is whether any stretch of cardiac mucosa indicates some level of foregoing insult to the gastro-oesophageal junction, or whether cardiac mucosa represents a physiological transition zone ubiquitously present between the distal squamous oesophagus and the oxyntic gastric mucosa. Numerous studies have dealt with this issue and the arguments exchanged will not be recapitulated here. Important for our current discussion is that recent studies have revealed an expansion over time with regards the length of cardiac mucosa in patients with chronic reflux complaints[94]. This could indicate that an initially physiologic mucosal transition zone physically expands in response to chronic insult. Over time such patients may also develop foci of intestinal metaplasia. These patients need not ever show the development of bona fide Barrett oesophagus in the native oesophagus. In fact, the

recent prospective BEST3 trial has highlighted that some 25% of patients with reflux complaints show intestinal metaplasia in the proximal stomach without presence of Barrett oesophagus[95]. The cancer risk incurred by this mucosal alteration, if any, is completely unclear at present. This lack of knowledge highlights the need for a defined risk profile to prospectively study this patient population and understand the risk for cancer progression at the gastrooesophageal junction. Ideally, these data should then be compared to studies focussing on progression of Barrett oesophagus proximally and of IM in the non-junctional stomach more distally.

MOLECULAR INSIGHTS

The last decade has brought ground-breaking progress regarding the scientific analysis and understanding of whole genome assessment of a broad range of diseases. A focus is now on the integration of genomic, epigenomic and transcriptomic data with clinical features and, especially in case of oncological conditions, outcome variables. With regards to the analysis of precancerous conditions, there is more abundant data on Barrett oesophagus compared to gastric IM due to the easier endoscopic detection and hence opportunity for longitudinal surveillance with overall less impact of sampling error.

Biopsies taken from segments of Barrett oesophagus adjacent to a cancer show clonal differences in DNA content, pattern of LOH and DNA mutations, typically in genes such as *CDKN2A* and *TP53*[96]. Genetic diversity between such spatially distinct samples is a predictor of further progression from Barrett metaplasia to invasive neoplasia[97]. In 2010, Leedham *et al.* investigated regions of LOH as well as *CDKN2A* and *TP53* point mutations on an individual crypt level in samples from Barrett oesophagus and concluded that heterogeneity within Barrett lesions originates from multiple independent clones resulting in a polyclonal mosaic[98]. Ross-Innes *et al.* analysed 73 samples from a 10cm Barrett oesophagus that had been collected over a period of three years during which the patient showed progression from gastric metaplasia to intestinal metaplasia, low grade and high grade dysplasia to, finally, intramucosal cancer. A polyclonal and thus heterogeneous genomic landscape of Barrett oesophagus explains the lack of mutational overlap between samples from oesophageal adenocarcinoma and adjacent Barrett metaplasia[99]. Non-dysplastic Barrett segments from patients who do not show any further neoplastic progression display a profile distinct from those who are classified as progressors, including a higher rate of pathogenic mutations, most obvious again for *TP53*[100]. In some cases, *TP53* mutation can lead to a whole genome doubling and a more rapid generation of genetic instability predisposing to cancer[101]. Samples from IM shows more frequent CNAs in respective cancer genes compared to samples from non-goblet cell, i.e. gastric,

metaplasia[102]. It is yet unclear which factors are responsible for these differences, but it is likely that the molecular pathways involved depend on multifactorial input.

A single study has profiled gastric intestinal metaplasia through targeted gene panel resequencing and methylation arrays and found that gastric intestinal metaplasia revealed a significantly lower mutation burden compared to mature gastric cancer with relatively few copy number alterations or epigenetic events[103]. A caveat to this work is that the intestinal metaplasia available for analysis was not purified before sample preparation, meaning low cellularity might have obscured bona fide genetic alterations. In general, however, the genetic constitution of gastric intestinal metaplasia is much less resolved compared to Barrett intestinal metaplasia. It remains unclear, for example, whether shared phenotypes are a consequence of shared (epi)genotypes.

Oesophageal adenocarcinoma displays high mutational burden of around 10 single nucleotide variations (SNVs) per megabase[99]. It is a genetically a very heterogenous disease with only a small set of recurrently mutated genes. These driver genes include well-known tumour suppressors like *TP53* and *SMAD4*, as well as *ARID1A* and other members of the SWI/SNF chromatin remodelling complex[104,105]. Barrett oesophagus is also prone to epigenetic changes with hypermethylation of key tumour suppressor genes[106]. Oesophageal adenocarcinomas often show features of chromothripsis, a phenomenon of chromosomal shattering due to errors in chromosomal segregation during mitosis[107]. So-called breakage-fusion-bridge events define a further category of large-scale re-arrangements involving telomeric loss, chromosomal fusion, and disrupted separation during anaphase. A number of oncogenes were found to be amplified in oesophageal cancer as a result of such events[108]. A better understanding of the factors causing these catastrophic events will help to describe the sequential evolution from Barrett oesophagus to oesophageal adenocarcinoma.

Gastric cancer shows features broadly comparable to oesophageal adenocarcinoma[109]. Aneuploidy and copy number aberrations (CNA) are common characteristics, and *TP53* is the most commonly mutated gene also in gastric cancer (60-70%). Genes that inhibit cell cycle entry, such as *CDKN2A* (p16) and *CDKN1B* (p27), show reduced expression in nearly half of all gastric cancers, often due to promotor hypermethylation. In 2014, *The Cancer Genome Atlas* (TCGA) consortium presented comprehensive data on about 300 gastric cancers, with the integration of data from five different 'omics'-platforms resulting in the proposal of a new molecular classification of gastric cancer into four groups[110]. The first group is characterised by epigenetic hypermethylation and shows a strong association to EBV infection. The second group is positive for microsatellite instability (MSI), similar to the respective subgroup of colorectal cancers. The remaining tumours were divided into those with low mutation rate and low frequency of CNAs (so-called genomically stable or GS), and those with a high mutation rate and further related genomic changes (chromosomally instable, CIN)[110]. CIN

cancers represent more intestinal type and junctional cancers. The *Asian Cancer Research Group* followed a similar approach with more emphasis on the utilization of transcriptome data for stratification into also four distinct groups[111]. Importantly, these subtypes were associated with distinct clinical outcome.

The combination of genome-wide high throughput data and bioinformatic analysis methods provided a new tool to dissect the aetiology of GOJ cancers. Initial differential gene expression analyses suggested a separation into diffuse and intestinal type gastro-oesophageal cancer with the intestinal-type tumours further separating into proximal and distal cancers[112,113]. The most intriguing data has been published by the TCGA consortium in 2017[114]. The authors analysed a combined dataset of more than 500 gastro-oesophageal cancers including 90 oesophageal squamous cell cancers and 165 tumours classified as GOJ cancers. Both oesophageal and GOJ adenocarcinomas are represented within the four main molecular subgroups that have been established for gastric cancers, while oesophageal squamous cell cancers are a molecularly distinct entity[114]. Rare MSI and GS cancers mirror the small proportion of diffuse type or signet-ring cell cancers at the GOJ[115], but the vast majority are of the CIN phenotype. These data suggest that GOJ cancers as well as oesophageal adenocarcinomas represent molecular phenotypes in many ways mirroring those of distal gastric cancer, and that the distribution of these gastric molecular phenotypes within tumours arising at different locations is the reason for the heterogeneity of previous studies. This is in line with our data on the expression profile of 84 GOJ and 23 non-junctional gastric cancers[116]. Whilst there was no correlation of the gene expression with clinical characteristics (including tumour location and the presence of Barrett oesophagus), GOJ cancers separated into three independent molecular groups with different clinical outcome by unsupervised clustering, with the same three groups being present in the remainder of the stomach. Whole genome sequencing data on a subset of these patients further corroborated these results[116]. Thus, it seems that we can find similar molecular phenotypes of adenocarcinoma across the upper GI tract, but that the distribution of these phenotypes varies depending on cancer location. It is likely that not only the imminent risk factors (e.g. *H. pylori*, acid or bile reflux), but also the interaction with the local microenvironment is responsible for the biology and differential clinical behaviour of these tumours.

One option to investigate the impact of specific risk factors on tumorigenesis is the study of mutational signatures, i.e. the pattern of base substitutions in a trinucleotide context across the genome[117,118]. Some of these signatures can be linked to tumour aetiology and certain risk factor exposure, e.g. a signature associated with exposure to ultraviolet light in melanomas[117]. Gastric cancers show commonly signatures associated with mutations in BRCA genes as well as frequently a signature associated with age[118]. In oesophageal adenocarcinomas, a common mutational

signature of T:A>G:C transversions in a CTT setting has been suggested to be associated with a mutation pattern caused by acid or bile exposure in the context of gastroesophageal reflux[104,105,108]. Unbiased stratification of oesophageal cancers based on the mutational signature profile also resulted in three subgroups that show different biological features and clinical characteristics[119]. It is likely that the exposure to different risk factors (e.g. bile reflux, *H. pylori* infection) in the upper gastrointestinal tract has an impact on these signature profiles. It would be insightful to generate additional data from well-annotated preneoplastic conditions to better understand evolutionary pathways to cancer and their intersection with clinical risk profile.

EVIDENCE FROM ANIMAL MODELSUnderstanding GOJ carcinogenesis has been severely restricted by the absence of a tractable pre-clinical model which might assist in providing new insights into the biology of an inflammation-driven metaplasia to dysplasia sequence, and the factors that drive inflammation-induced carcinogenesis. To date, there are few genetic mouse models available which lead to the generation of metaplastic and dysplastic tissue at the GOJ.

Data from an embryonic mouse model suggest that Barrett oesophagus might arise from residual embryonic cells (REC) in the oesophagus[120]. In this study, p63 knockout mice were shown to develop a Barrett-like metaplasia in the squamous forestomach, and retrograde growth of a population of *car4*-expressing cells located at the squamo-columnar junction was proposed as the origin of the metaplasia. In this p63-deficient mouse model, intestinal metaplasia develops during embryogenesis[120]. P63 is crucial for stem-cell self-renewal in the stratified squamous epithelium, and thus p63 knockout embryos provide evidence for the role of embryonic stem cells in metaplasia development at the transition zone to squamous epithelium[121]. In this hypothetical model, RECs represent an opportunistic cell type that rapidly expand upon reflux-induced tissue damage. In human tissue, the majority of oesophageal adenocarcinoma do not express p63, in contrast to strong p63 expression by oesophageal squamous cell carcinomas[122].

The most recent report by Jiang *et al.* proposed that Barrett oesophagus arises from a group of distinct basal progenitor cells (p63+KRT5+KRT7+) in the transitional squamous epithelium, immediately adjacent to the gastric cardia at the GOJ[123]. The authors used multiple transgenic mouse models and lineage tracing strategies to show that this basal cell population can serve as a source of progenitors for the transitional epithelium. Upon ectopic expression of CDX2, the

transitional base cells differentiate into intestinal-like epithelium including goblet cells, and hence reproduce core features of Barrett metaplasia.

The most frequently used mouse model invokes a different strategy by manipulating the microenvironment via oesophageal overexpression of interleukin-1β, thereby simulating the inflammatory reaction caused by acid and/or bile reflux. This approach mimics the inflammation-metaplasia-dysplasia-cancer sequence observed in humans in the context of a transgenic mouse model[124]. These transgenic mice exhibit esophagitis progressing to Barrett oesophagus at the GOJ within 4-6 months and spontaneously to cancer with older age. Progression is accelerated in the presence of bile acid and/or carcinogens, and by a high fat diet (HFD)[91,125], generating an inflammatory microenvironment that accelerates tumorigenesis. This mouse model has provided fundamental insights into the pathogenesis of Barrett oesophagus, and offers a molecular basis for an emerging paradigm shift regarding the cell of origin of Barrett oesophagus and oesophageal adenocarcinoma (**Figure 3**)[126]. Similar to the concept proposed in the original paper by Dr Norman Barrett himself in 1950[127], the murine model of Barrett oesophagus and oesophageal adenocarcinoma suggests that metaplastic lesions originate from progenitor cells in the gastric cardia, which over time expand in the direction of the GOJ and the squamous oesophagus and are strongly associated with the development of dysplasia, in both human and murine Barrett oesophagus. The trigger for activating progenitors in the gastric cardia may be chronic inflammation of the cardia associated with GORD or, possibly, *H. pylori*. It is thought that prior to the development of Barrett oesophagus, a regenerative cell lineage appears in the oesophagus that expresses TFF2 and CDX2, followed later by the appearance of intestinal (goblet cell) metaplasia[128–132]. In *L2-IL1B* mice, conditional activation of Notch signalling in $LGR5^+$ progenitor cells reduced the maturation of goblet cells, increased crypt fission, and accelerated the development of tumours at the squamo-columnar junction. Conversely, mice with inhibited Notch signalling in $LGR5^+$ cells display increased goblet cells, reduced crypt fission, and fewer tumours[91]. IM itself might thus not be a typical precursor lesion. Given the stable nature of Barrett oesophagus, it probably reflects the expansion of some sort of an altered stem cell population due to alteration of the stromal niche factors.

Although there are some discrepancies in the data generated with the various mouse models addressing the origin of Barrett oesophagus, they share evolutionary mechanisms including the final presence of a high diversity of cell lineages, and a strong selective pressure exerted by the chemically modified (acid/bile) microenvironment that allows Barrett oesophagus cells to gain a fitness advantage over the normal squamous epithelium. Of special interest is evidence in mice that bile-acid reflux after a surgical oesophago-duodenal anastomosis leads to expansion of epithelial cells expressing CDX2 resulting in goblet cells only at the gastro-oesophageal junction and not elsewhere

in the oesophagus. This suggests that the cell of origin must be located within the area of the gastro-oesophageal junction, especially as the squamous oesophagus, which was also exposed to bile-acid reflux, contains neither cell type.

Overall, robust data from the *L2-IL1B* mouse model suggest that a progenitor/stem cell population within the gastric cardia expands during the development of Barrett oesophagus[124]. Cardia progenitor cells possess the ability to differentiate into a secretory cell lineage[91], and they expand into the oesophagus to give rise to Barrett metaplasia and dysplasia. Growing evidence from the mouse models points to an important role of the microenvironment in triggering many of the earliest events of tumour initiation[91,125,133,134]. Like stem cells residing in a niche that maintains these cells in a stem-like state, tumour initiating cells also require a dedicated microenvironment to control their self-renewal and undifferentiated state. In oesophageal carcinogenesis chronic inflammation promotes the proliferation and survival of malignant cells by subverting innate and adaptive immune responses and altering the response to hormones and chemotherapeutic agents. In the *L2-IL1B* mouse model, IL8 induced Barrett oesophagus and oesophageal adenocarcinoma in part through recruitment of immature myeloid cells[125,133]. Apart from cancer cells and infiltrating immune cells, the stromal microenvironment of tumours includes a mixture of mesenchymal cells, comprised mostly of carcinoma associated fibroblasts, a cell type that closely resembles normal myofibroblasts present in the gastrointestinal mucosa. Activated fibroblasts contribute to tumorigenesis by enhancing proliferation and the tumour initiating capacities of tumour cells, and indirectly by recruiting and polarizing cells of the adaptive and innate immune system towards a tumour-promoting phenotype[133,135,136]. An additional role was recently attributed to factors mediated by visceral adipocytes.

Although animal models enhance our understanding of carcinogenesis at the GOJ, these models do not diminish the need for human data to elucidate the complex interaction of the oesophagogastric epithelium with the local microenvironment in different parts of the upper gastrointestinal tract.

SUMMARY AND CONCLUSION

For decades, epidemiological studies have tried to define the origin of adenocarcinomas at the gastro-oesophageal junction. These efforts were often hampered by use of varied clinical definitions and often lacked meaningful insight into underlying biology of these tumours. Recent advances in next generation sequencing shed further light on this conundrum and it has been suggested that upper gastrointestinal adenocarcinomas are all of a similar nature, whilst still comprising different phenotypes. Although presence of each phenotype is not dependent *per se* on tumour location, the

relative proportion of molecular phenotypes (a phenotypic mosaic) varies along the upper gastrointestinal tract. The current understanding is that these phenotypes are not only driven by specific risk factor exposure, i.e. mainly *H. pylori* and acid or bile reflux, but also defined by factors pertinent to the local microenvironment. This includes the epithelial or rather glandular architecture and cell composition, the presence and nature of stem cell populations, and the interaction between epithelial, and stromal mesenchymal and immune cells. An altered stem cell population at the gastro-oesophageal junction expands due to chronic injury from the stomach or oesophagus and multiple clonal populations arise within a specialised niche over time. With the development of metaplasia, these GOJ stem cells provide a successful phenotypic adaptation to recurrent tissue damage. Recurrent clonal selection from stem cells within the gastric cardia, however, ultimately drives clonal progression to cancer. Chronic injury (induced by chemical of infectious agents) and inflammation in a gastric or oesophageal microenvironment emerges as a major factor in transformation of the GOJ-derived stem cells and progression to cancer. Clearly then research focus shifts towards understanding the contextual drivers which determine whether columnar metaplasia behaves as a successful and protective adaptation to damage, or a lesion that heralds increased cancer risk. A better understanding of the complex processes that lead to adenocarcinomas of different phenotype will not only help to identify new targets for tailored cancer treatment, but also to design new strategies for individually stratified early detection and prevention of upper gastrointestinal cancers, including those arising at the GOJ.

Practice Points

- Intestinal metaplasia arising within the upper GI tract should be considered as a risk indicator and endoscopic surveillance should be discussed in line with international and national guidelines.
- Patients with cancer at the gastro-oesophageal junction should be checked for *H. pylori* infection and their history of reflux symptoms.
- Systemic treatment of cancer of the gastro-oesophageal junction should follow the same pathways as gastric cancer therapy due to the shared molecular background.
- New molecular classifications do not yet allow guidance on clinical treatment decisions, but this is likely to be implemented in coming years.

Research Agenda

- There is need for a comprehensive molecular and genetic characterisation of intestinal metaplasia at the cardia (in comparison to IM in the remainder of the stomach), similar to the data that has been generated for Barrett oesophagus.
- This includes molecular phenotyping of IM, factoring in risk factors and influences by the local (cellular) microenvironment.
- This knowledge should be implemented in longitudinal, prospective risk stratification studies, with a special focus on patients with IM at the cardia and no Barrett oesophagus.
- Studies on mutational signatures might facilitate the understanding of the association with and the interplay of specific risk factors.
- There is need for a validation of the clinical implication of molecular classifications of gastric cancer, especially in view of treatment allocation of targeted therapies.

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CONFLICT OF INTEREST

None of the authors have any financial or other conflict of interest to disclose with regards to the content of this manuscript.

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FIGURE LEGENDS

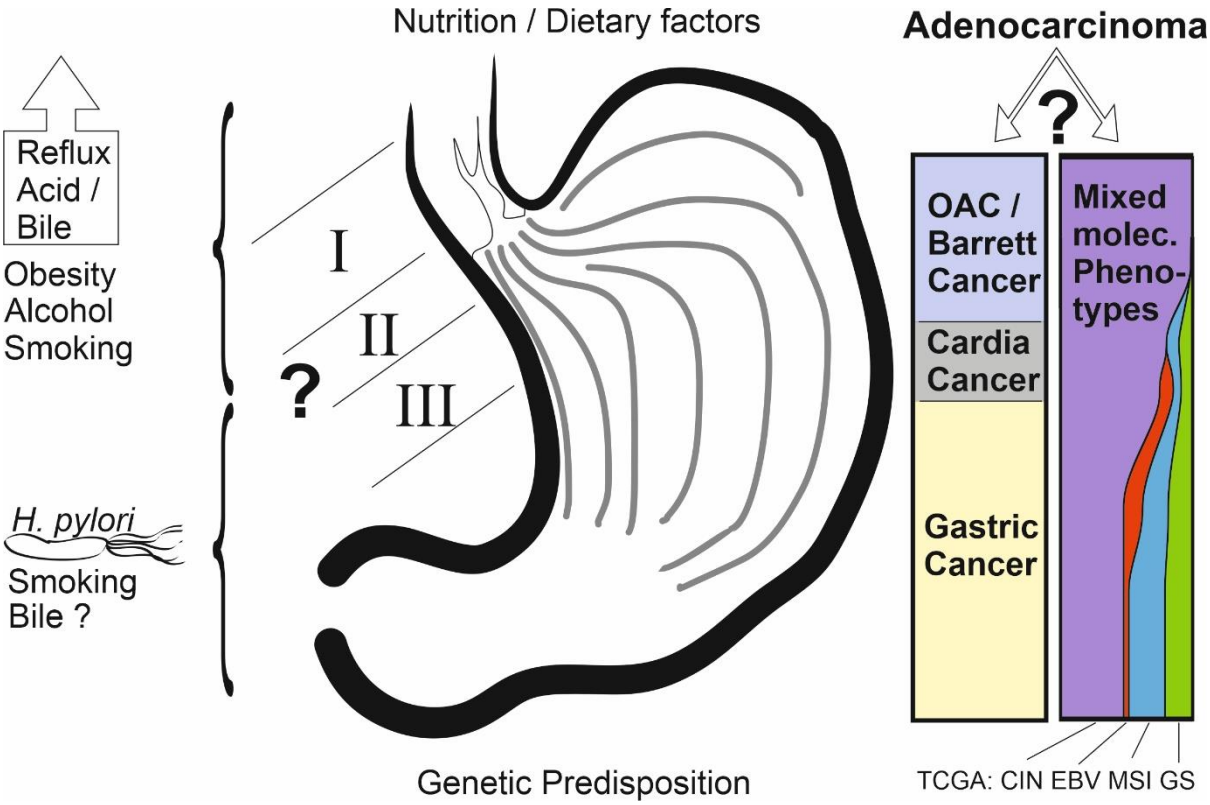

Figure 1: Risk factors involved in upper gastrointestinal carcinogenesis

Epidemiological studies showed an association of specific risk factors to cancer at the gastro-oesophageal junction (GOJ). Gastro-oesophageal reflux and associated factors, e.g. obesity, are associated with oesophageal cancers and those proximal at the GOJ, H. pylori infection with non-junctional gastric cancer and tumours located distally at the GOJ. Alimentary factors and the genetic predisposition play a further role in modifying the individual cancer risk. From a molecular perspective, there is still debate if upper gastrointestinal adenocarcinomas comprise of location-specific entities (i.e. oesophageal, cardia and gastric cancer) or if rather the combination of shared molecular phenotypes varies by tumour location (TCGA: The Cancer Genome Atlas Consortium, CIN: chromosal instability tumours, EBV: Epstein Barr virus associated tumours, GS: genomically stable tumours, MSI: microsatellite instability positive tumours [114]).

Figure 2:

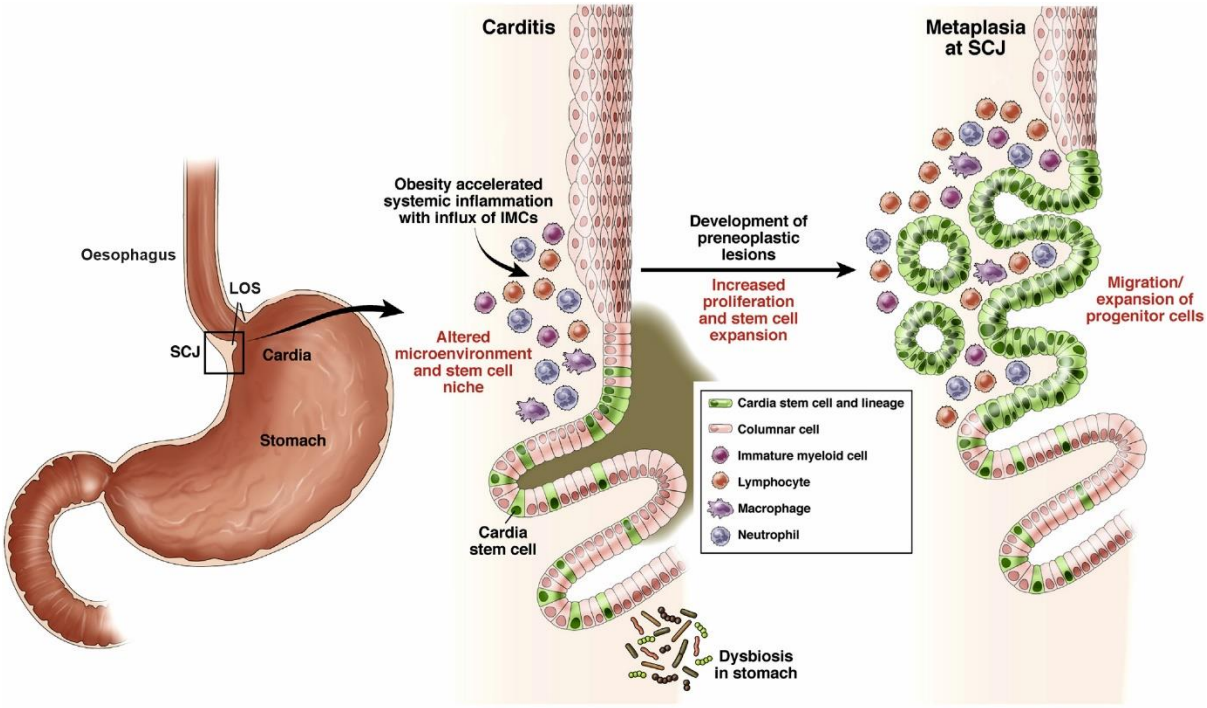

Figure 3: Microenvironment and stem cell expansion in Barrett oesophagus.

In a proposed model of Barrett oesophagus, the proximal extension of original cardiac mucosa originates metaplasia in the oesophagus. In obese patients, accelerated flux of immune cells and expression of cytokines, as well as increase intra-abdominal pressure with an increased exposure of bile and gastric acid to the lower oesophagus sphincter (LOS) likely results in an altered microenvironement at the squamo-columnar junction (SCJ) that induce a lengthening of the cardiac mucosa through an expansion of adjacent cardiac glands. (Figure from [126]).