THE PATHOLOGISTS' EYES ON FOREGUT: HISTOPATHOLOGICAL RELATIONS IN THE EXPERIMENTAL AND ROUTINE DIAGNOSTICS

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1. BACKGROUND

1.1 Chronic gastroesophageal reflux develops esophageal cancer

Chronic gastroesophageal reflux disease (GERD) carries an increased risk for development of esophageal mucosal injury, columnar metaplasia (Barrett's esophagus – BE), dysplasia and adenocarcinoma of the esophagus (EAC). These alterations are provoked by the reflux juice reaching the esophageal mucosa from the stomach which usually contains mixed acidic and bile components.
To model the effect of potential carcinogenic mixed reflux juice there are appropriate surgical methods (e.g. side-to-side fashion esophago-duodenostomy) which provide a suitable model for reflux-induced esophageal pathologies without the need for additional carcinogen administration.

1.2 BE is a GERD-generated columnar metaplasia above the gastroesophageal junction

Columnar lined esophagus became widely used as BE since 1957 due to Norman Barrett. According to the original definition of BE, metaplastic columnar mucosa occurs in the squamous lined esophagus above the cardiac region caused by damage of biliogastric juice during GERD. Histopathologically, metaplastic columnar mucosa was subdivided into fundus (oxyntic), cardiac and intestinal epithelium according to Paull et al. Nevertheless, from that time data showed that only intestinal metaplasia has neoplastic transformation capacity and BE has gained significance after having been identified as a facultative premalignant condition of EAC together with high coincidence of GERD.
According to recent data there are several possible routes which able to characterize the genesis of columnar metaplasia in the lower esophagus. Perhaps the most widely known pathway is the creeping form when columnar mucosa grows up to the esophagus replacing squamous epithelium. This idea supposes to testify the overgrowth of gastric mucosa by reflux induced glandular hyperproliferation. Another restitution hypothesis is decribed when the reflux-damaged squamous epithelium replaced by glandular and subsequently foveolar epithelium as a part of regeneration of esophagus. This process can originate from the altered differentiation of duct stem cells in the submucosal cardiac glands. The latest hypothesis involves multilayered epithelium (MLE) which is biphenotypic. In MLE the basal part of the surface mucosa is squamous but the upper layer is already differentiated into mucous foveolar cells containing abundant neutral or acidic mucin. Sometimes even cilia are also seen reminiscent to respiratory epithelium. In the upper mucinous layer goblet cells can be frequently identified.

1.3 Metaplasia-dysplasia-carcinoma sequence in BE

Debates about the genesis of columnar metaplasia would have been meaningless if BE is not playing a role in the genesis of EAC. In this process the first critical point is the presence of dysplasia. Several study aimed to characterize the genetic progression of metaplasia-dysplasia carcinoma

sequence in BE. Many genes and their encoded proteins can be involved regarding to cell cycle regulation (e.g. Cyclin D1, CerbB2, EGFR, Rb, P53, P16, P38, P27), apoptosis mediation (eg. Bax, Bclx, NfkB, Cox2), invasion (e.g. Catenins), angiogenesis (eg. VEGF) and cytokine pathways (e.g. TNFα). Although these gene and protein alterations can be arranged in order of progression of BE - carcinogenesis these finding are hardly used generally for each BE-case.

1.4 Histological aspects of dysplasia in foregut intraepithelial neoplasia

Presence of dysplasia in metaplastic Barrett's mucosa and gastric intestinal metaplasia is the most reliable histopathological predictor for ongoing carcinogenesis but there are several pitfalls in the precise evaluation of dysplasia giving a marked interobserver variation. Although modified Vienna classification may decrease the interobserver variation other additional tissue biomarkers can also contribute for accurate assessment of dysplasia. For instance the proliferatory Ki67 labeling and mutant P53 intranuclear accumulation of dysplastic glands may have diagnostical implications.

1.5 Foregut premalignancy and early malignant change: diagnostic challenges

For the accurate diagnosis of BE standardized endoscopic sampling should be used. From gastric metaplasia also multiple forceps biopsies should be taken, however, in early esophago-gastric neoplasia there are methods available not just for diagnostic but for therapeutic implications as well. One of these methods is the endoscopic mucosal resection (EMR).

EMR of superficial early cancers of the upper gastrointestinal tract is a standard technique in Japan and is currently increasingly used in other developed countries. Early stage esophago-gastric cancer (EGC) is defined as the state when the tumor invasion is confined to the mucosa or submucosa irrespective of the presence of lymph node metastasis. The reliability of the histological results of forceps biopsy sampling with regard to the entire lesion and a precise diagnosis is therefore essential for a correct therapeutic decision. The aim of EMR is the complete removal of the diseased mucosa by resection through the middle or deeper part of the the submucosal layer. Moreover, the potential use of EMR as a diagnostic tool has been suggested.

2. AIMS

I. The main purpose of the experimental study was to investigate the incidence of GERD-induced malignoma formation due to the duodeno-esophageal anastomosis. After the identification of simultaneous esophageal squamous cell carcinoma and mucinous EAC formation in one of the animals we aimed to perform a detailed analysis of the immunophenotypes and simultaneous activation of more than one possible carcinogenetic pathway during the malignant transformation of the exposed esophageal tissues.

II/1. In the human BE study a standardized histopathological analysis was performed focusing not only on the SIM but also on the presence of additional mucinous cardiac and superficial mucous glands and the role of fundic and pancreatic acinar metaplasia in the metaplastic process. Therefore, we aimed to present the results of histopathological processing according to the schemes of standardized and detailed histopathological investigation of endoscopic biopsies, focusing upon all tissue counterparts of the samples.

II/2. In the EMR study we aimed to assess the value of forceps biopsy sampling in establishing the correct diagnosis revealed by EMR and to evaluate not just the therapeutic but also diagnostic efficacy of EMR.

3. MATERIALS AND METHODS

3.1 Collection of materials

3.1.1 Animals and design of experimental surgery

For the experimental GERD study 31 male Sprague-Dawley rats (average weight 250 g) were used. A longitudinal midline laparotomy was performed, and a side-to-side fashion esophago-duodenostomy was made with 7/0 atraumatic non-absorbable interrupted stitches to join the duodenum to the esophagus. This procedure permits chronic reflux of mixed duodenal and gastric contents into the esophagus through the site of the anastomosis, while the normal stomach function and normal nutritional status are preserved. The rats were sacrificed 30 weeks after surgery by diethyl ether inhalation and the abdomen and thorax were opened. The esophagus and the stomach with the anastomosed duodenum were removed in continuity, longitudinally opened, and spread on a cork plate for macroscopic examination.

3.1.2 Patient's collection for human BE study

For human BE surveillance study patients were collected consecutively. Between 2004 and 2007, 3809 endoscopic biopsy samples of 826 consecutive patients with endoscopically suspected esophageal metaplasia have been evaluated. The macroscopic extension of the metaplastic mucosa was established by detailed description. Apart the circumferential and maximal extension of the metaplastic mucosa we recorded the extension and relative location and of all isolated and flame shaped lesions. The top of the gastric folds and the distal end of the esophageal palisade veins were used to determine the level of the GEJ. All endoscopic examinations were recorded to video or DVD. On this basis we formed patient groups with short (<3 cm) and long segment (≥3 cm) metaplasia. Biopsies were taken according to the internationally accepted method: four quadrant biopsies from every two centimeters of circumferential metaplasia. Non-circumferential - isolated or tongue-like -

metaplastic areas were also sampled according to their length. At least one biopsy was taken from every two centimeters. Samples were sent for histology in different containers.

3.1.3 Sampling of EMR

For the EMR study both forceps biopsy and EMR samples were received from the same lesions. Fifty-six subjects with sessile gastric polyps of epithelial origin, at least 0.5 cm in diameter, and not associated with polyposis syndromes, were included. The EMR were performed in the Jávorszky Hospital, Vác, and the First Department of Medicine at the University of Szeged in the period 2000–2006. High-resolution electronic endoscopes (EG 410 HR or EG 205 WR 5; Fujinon, GIF Q140; Olympus, Tokyo, Japan) were used. Pharyngeal anesthesia was induced with lidocaine, and sedation with midazolam. The precise margins of the lesions were established by chromoendoscopy spraying with 0.1–0.5% indigo carmine dye (Reanal, Budapest, Hungary). Two to eight (average: six) samples were obtained from each lesion by ordinary forceps biopsy (Maxum; Wilson-Cook, Winston-Salem, NC, USA). Endoscopic ultrasonography (EUS) (GF-UM-130; Olympus) was carried out in all cases. EMR was carried out with inject-and-cut or with cap-fitted methods. After chromoendoscopy, adequate marking surrounding the lesion was achieved with high-frequency electrocautery (PSD 10; Olympus). 2–10 mL normal saline solution was injected into the submucosal layer beneath the lesion to raise it (NM 200U; Olympus). The lesion was resected with a snare (SP-5U-1 or SD-17L; Olympus) with high-frequency current. After EMR, the patients participated in sucralfate and PPI therapy. Aspirin treatment, discontinued 7 days before the EMR, was reintroduced 7 days after the procedure. None of the patients received warfarin. All complications occurring during polypectomy or in the subsequent observation period were documented. Resected specimens were extracted, washed in normal saline, oriented and marked.

3.2 Routine histopathology

Either parts of the experimental rat specimen or tissue samples from endoscopically evident columnar lined esophagus, forceps biopsies and EMRs were received and fixed in 4-10% neutral buffered formalin in phosphate-buffered saline, while the other part of rat esophagus was stored at -80 ^{o}C. In the animal model esophagitis, dysplasia, BE, EAC and ESCC were classified according to standard classification techniques. In the human BE study each tissue sample underwent detailed morphological assessment based on histopathological dataset. Proper clinical data listed in the endoscopy report were also required. Each case was examined at least 8 levels. Formalin-fixed and paraffin-embedded blocks were used for light microscopy, using hematoxylin-eosin (HE), periodic acid Schiff - alcian blue (PAS–AB, pH=2.5) and occasionally Gomori's aldehyde fuchsine – alcian blue (GAF–AB) staining for the identification and subtyping of intestinal metaplasia. Dysplasia was assessed according to the modified Vienna classification. Slides were coded and re-evaluated blindly by two independent pathologists.

3.3 Immunohistochemistry

For immunohistochemistry, 4 µm sections were cut from the same paraffin block, placed on special sylanized slides dry upright overnight at 56°C temperature in incubator, dewaxed in xylene and rehydrated in decreasing concentrations of alcohol. Slides were incubated in 3%H_2O_2 in methanol for blocking the tissue endogenous peroxidase activity.

For the experimental GERD study tissue sections were identified with cyclooxygenase-2 (Cox-2), C-erbB2 and cyclin D1 antibodies. In each case, positive and negative controls were included. In BE cases suspected for dysplasia routine immunohistochemical investigation was performed usinusing P53 and Ki67 antibodies. In EMR study neuroendocrine or gastrointestinal stromal origin was proved using Chromogranin A and C-kit antibodies. For visualization DAB solution was used under microscopic controlling of brown coloration. Slides were counterstained with hematoxilin than dehydrated in increasing concentrations of alcohol cleared in xylene and coverslipped. The coded sections were analyzed by two independent investigators. The ratio of positive/negative cells was calculated by inspecting 10 consecutive high-power fields.

3.4 Genetic analysis

In the experimental GERD study one part of the esophagus was sent for rapid RNA isolation and quantitative real-time polymerase chain reaction (Q-RT-PCR). For the RNA isolation, the proximal and distal parts of the esophagus were separated. The changes in the mRNA abundance of these genes were followed by means of Q-RT-PCR to compare the gene expression changes caused by the experimental GERD. For controls, 6 age-matched healthy rats were sacrificed, their esophagus was removed and cut into two parts, and the abundances of p53, cyclin D1 and cox-2 mRNAs were detected. The Q-RT-PCRs were performed with iQ Supermix (Bio-Rad Laboratories) in an iCycler (Bio-Rad Laboratories).

3.5 Ethical considerations, patients' data management, statistical analysis

The experiments were performed in accordance with the National Institutes of Health Guidelines (Guide for the Care and Use of Laboratory Animals). The study protocol was approved by the Animal Welfare Committee of the University of Szeged. Data of patients were managed confidentially according to the local ethical legislations.

Genetic data were evaluated via descriptive statistics. For data analysis chi-square and Fisher exact tests were performed using STATA software (version 9.0). The sensitivity and specificity of the forceps biopsy procedure for diagnosing neoplastic lesions was provided with 95% confidence intervals ($p<0.05$).

4. RESULTS

4.1 Histopathological changes and genetic alterations of neoplastic esophageal mucosa due to experimental GERD

48% of animals who underwent surgery showed signs of the chronic GERD, including basal cell hyperplasia, acanthosis and hyperkeratosis. In the animals with GERD, malignoma formation was evident in 4 cases (25%), and detailed histology analyses were performed on these tissue samples. Macroscopic and microscopic findings for case 29 are further detailed. The dissection of the esophageal specimen 29 showed typical GERD features as thickened wall and marked irregular folds with a typical cobblestone appearance, extending from the mid-esophagus to the angulus of the stomach. The appearance of the larynx and duodenum was normal. In the stratified squamous epithelium of the mid-esophagus, typical reflux-associated signs were present. The lower part of the esophagus exhibited ulcerated squamous cell carcinoma with invasive foci of keratin pearls. The adjacent squamous epithelium was dysplastic, ranging from mild dysplasia to carcinoma in situ, where the basal layer was already replaced by skipping metaplastic columnar mucosa. PAS–AB and GAF–AB staining revealed the acidic sulfomucin content of the metaplastic goblet cells, indicating type III SIM. At the squamocolumnar junction, microscopic mucinous adenocarcinoma (maximum diameter 2 mm) invading the submucosal region was identified. The tumor was surrounded by dense mononuclear inflammation and mastocytosis at a distance of 1 mm from the squamous cell carcinoma. Predominantly the basaloid part of the dysplastic squamous epithelium and squamous cell carcinoma cells exhibited Cyclin D1 expression (nuclear positivity in 35% of all the squamous cells) and P53 protein accumulation (nuclear positivity in 50% of all the squamous cells) with a low expression of Cox-2 (less than 10% of all the glandular cells) and negative C-erbB2 staining. The SIM and mucinous adenocarcinoma cells displayed exclusively diffuse Cox-2 (90% of all the glandular cells) and weak focal C-erbB2 (5%, restricted to the dysplastic glandular cells) expression. The adenocarcinoma cells did not exhibit Cyclin D1 expression or P53 protein accumulation. The expressions of these genes were significantly increased; for cyclin D1, a 9.08-fold elevation was observed, while the p53 and cox-2 gene expressions were increased 1.61-fold and 2.45-fold, respectively, as compared with the non-treated control tissue samples. These gene expression changes are correspondent with the topographic immunoexpression data.

4.2 Tissue counterparts, frequency and distribution of metaplastic columnar mucosa in BE patients

The total number of samples was 3809 of 826 (432 male and 394 female) patients. Multiple samples obtained from one patient are considered as one case since they represent the whole metaplastic area of BE. The overall mean age was 55.6 (SD: 14.7) years; 54.5 (SD: 15.1) and 56.7 (SD: 14.3) years in the female and male groups, respectively ($p=0.04$).

During evaluation of the specimens all counterparts were considered including alterations of the squamous epithelium, subtyping of metaplastic glands and evaluation of dysplasia with the assessment of stromal elements. As mentioned above tissue samples representing the metaplastic area of each patient were summarized as one case. Since the cases frequently contained more than one type of mucosa, the glands were classified as either ’main’ or ’associated’ metaplastic lesions. The ’main’ metaplasias are considered to be obligatory parts of the metaplasia; at least one of them should exist in each sample (as cardiac, oxyntic (fundic), oxintocardiac or SIM).

As far as ’associated’ glands are considered, they are facultative members of the metaplastic mucosa as pancreatic acinar and ciliated metaplasias together with superficial mucous glands. While pancreatic acinar cells have typically zymogene granules, ciliated metaplasia reminiscent to respiratory pseudostratified epithelium and sometimes adjacent MLE can be also noted.

As well as superficial mucous glands are considered, they should be distinguished from cardiac glands since the cytoplasm of superficial mucous glands is much lighter. In addition to containing more lucent neutral mucin, superficial mucous glands are arranged in a typical crowding (back to back) architecture in the lamina propria and they never reach the luminal surface.

Numerically, only 4.1% (n=34) of all cases contained exclusively SIM. Those cases, which contained SIM (n=177; 21.5%) showed cardiac-fundic glands as well. The other cases (n=615; 74.4%) contained cardiac-fundic mucosa without SIM. Samples contained superficial mucous glands, pancreatic acinar and ciliated metaplasia accounting for 24% (n=198), 14.9 (n=123) and 0.2% (n=2), respectively. As well as the distribution of main and additional metaplasias considered, pancreatic acinar metaplasia was found near cardiac-fundic metaplasia (94/123), while SIM coexisted with superficial mucous glands (103/198 superficial mucous gland cases; $p<0.001$). Low grade dysplasia (n=51;6.2%) and high grade dysplasia (n=9; 1.1%), confirmed also by the gradually elevating P53 and Ki67 intranuclear immunolabelling, were found mainly at SIM (37/51; 9/9) with male preponderance (3:1 at low grade, 2:1 at high grade dysplasia). Helicobacter pylori infection was confined to only cardiac foveolar mucosa (n=17; 2.1%) without dysplasia. Short segment BE gave the vast majority of cases accounting for 95%. While cardiac mucosa represented both short and long segment cases, oxyntic mucosa was found mainly in short segment, and SIM in long segment BE, respectively ($p<.000$).

At more than half of the cases (61%) there was no additional metaplasia present, in the rest of the samples PAM rather coexisted with short segment, and Schaffer’s glands with long segment cases, respectively. Long segment BE showed a markedly dominance of dysplasia ($p=0.07$). Pancreatic acinar metaplasia was mainly associated with cases negative for dysplasia (103 of 123 pancreatic metaplasias), but low grade dysplasia cases contained superficial mucous glands (26/51). Presence of the superficial mucous glands predicts SIM without dysplasia with an odds ratio of 6.99, together with dysplasia of 3.98, respectively. The pancreatic acinar metaplasia decreases the odds of SIM turning up in the sample (odds ratio of 0.45). Both cardiac and SIM predict dysplasia with an odds ratio of 3.73 and 9.75, respectively.

4.3 Histopathological comparison between forceps biopsy and EMR

The histopathological analysis of the resected specimens revealed neoplastic lesions in 34 cases, including seven EGC, and there were hyperplastic-inflammatory lesions in 21 cases. In one case, the histological examination of the resected specimen was not informative owing to a thermal injury. Complete agreement between the previous histological results of the forceps biopsy samples and the resected specimens was seen in only 76.7% of the lesions. Altogether, the sensitivity and specificity of the forceps biopsy procedure for diagnosing neoplastic lesions were 87.5% (95% CI = 76.0–98.9%) and 65.2% (95% CI = 45.7–84.7), respectively. Because of their great significance, we have highlighted the EGC cases (intramucosal borderline neoplasia according to the modified Vienna classification). Seven EGC were diagnosed via the histological analysis of the resected specimens during this period: four females and three males, with a mean age at endoscopy of 68 (42–80) years. All seven patients had severe concomitant diseases. Only two of the seven EGC had been diagnosed previously by forceps biopsy. This means that complete agreement between the histological results on the forceps biopsy sample and on the ectomized polyp was seen in only 28.5% of the cases. In four EGC cases, the histological result on the previous forceps biopsy was adenoma with high-grade dysplasia in three cases, and hyperplastic polyp in one case. After careful histological study of the resected specimen, the latter case proved to be one of gastrointestinal stromal tumor (GIST), which was confirmed by C-kit (CD117) immunohistochemistry. The previous forceps biopsy had shown adenomas with high-grade dysplasia in three patients, whereas the EMR histology demonstrated well-differentiated intramucosal carcinoma in all of them. The previous forceps biopsy had indicated well-differentiated intramucosal carcinoma in one case, whereas the EMR histology revealed well-differentiated neuroendocrine tumor (carcinoid) with Chromogranin A immunostaining. The sensitivity of the forceps biopsy for the diagnosis of intramucosal borderline neoplasia was 85.7%.

4.4 Further clinical course of patients underwent EMR

The mean duration of hospital stay after the EMR was 7.6 days, and there was no mortality. No serious complications, such as perforation or massive bleeding necessitating surgical treatment, were encountered. Post-mucosectomy bleeding was observed in three of the 56 cases. All of them were treated successfully by endoscopic hemostasis, which was performed immediately after the EMR procedure in two cases, or 1 day after mucosectomy in one case. One patient required transfusion. After EMR, patients were followed up by endoscopy at 3, 6 and 12 months, and then once a year. The mean follow-up time was 38 months (6–72). EGC did not recur during the follow up. The EMR was considered to be complete in five patients. In two cases with well-differentiated intramucosal carcinoma, neoplastic glands were detected at the resection lines. In one of these cases, Nd YAG laser therapy was applied. In the other case, the patient underwent Billroth II resection, but cancer cells were not revealed in the resected specimen. This was probably due to the ‘burning effect’; the cancer cells at the resection margin were destroyed by the burning effect of the high-frequency current.

5. DISCUSSION

5.1 The carcinogenic potential of mixed acidobiliary reflux on the esophageal mucosa

In humans, most of the EACs arise in the BE where columnar cell metaplasia replaces the native squamous cell epithelium lining the distal esophagus. It is important to note that a gastric reflux alone has less potential to contribute in the Barrett metaplasia-carcinoma sequence. Premalignant lesion rather develops as a consequence of the chronic reflux of acid and bile secretions into the esophagus. It has been suggested that duodenal juice, i.e. bile, induces oxidative stress in the esophageal epithelium, thereby leading to chronic inflammation and then metaplasia. Bile salts may target several potentially interconnected pathways, leading to mucosal barrier impairment, and may alter the function of cells that they are likely to contact.. There is increasing evidence that supports the progression from metaplasia to dysplasia, and finally to EAC, indicating a disturbed cell cycle regulation in the reflux-induced carcinogenesis. An understanding of the nature of regurgitation-induced mucosal lesions presupposes the use of suitable experimental models. In our experiments, severe macroscopic, microscopic and molecular alterations were observed in nearly 50% of the cases after 30 weeks of chronic gastroduodeno-esophageal reflux. As in previous studies, we confirmed that duodenal juice without exogenous carcinogen administration can undoubtedly contribute to epithelial hyperproliferation and esophageal carcinogenesis.

In humans, the histopathology spectra of developing carcinomas mainly include EACs and adenosquamous carcinoma but there are also data about the role of duodenal bile-rich reflux in the pathogenesis of squamous cell carcinoma as well. Nevertheless, synchronous esophageal carcinoma formation with different histogenesis is highly unusual and only few data are available on the simultaneous initiation of divergent carcinogenetic pathways leading to the synchronous development of malignomas of different histological types in the same individual. The reason for this restriction is unclear, but Pera et al. demonstrated that esophageal tumors with squamous phenotype express p53 and cyclin D1 while the differentiation toward glandular-type epithelium is associated with decreased expressions of p53 and cyclin D1. Our data support and extend these findings. In this particular case, the squamous phenotype was associated with predominantly positive P53 and Cyclin D1 immunostaining, while the route toward BE and EAC differentiation was characterized by Cox-2 positivity. The EAC cells exhibited P53 and Cyclin D1 negativity, the most dramatic increase being observed in the level of expression of cyclin D1. Although gene expression data can not be linked to distinct tumors, this inverse change may be specific to EAC formation.

5.2 Different stages of maturation of metaplasia carry distinct experimental esophageal carcinogenesis

In our experimental GERD study it has been also observed that different cell types together with the cells in different stages of maturation (squamous primitive basal/spindle cells or mature keratinocytes and glandular metaplastic epithelium) can give rise to various reactions in the same field. The extensive investigation of Pera et al. demonstrated gradually increased expressions of p53 and cyclin D1, from papillary hyperplasia toward undifferentiated basaloid carcinoma, but gradually decreased expressions of p53 and cyclin D1 toward glandular differentiation. According to our working hypothesis is that two parallel pathways exist during the field effect of the duodenal content on the esophageal mucosa. The first, initiator event encourages the fast mucosal evolution of squamous basal (stem) cells to mature keratinocytes and/or metaplastic goblet cells. The second, carcinogenic event can act on different stages in the maturation of the promoted cells, resulting in tumors that have different gene expressions and phenotypic appearances. The combination of these pathways may result in various carcinomas, as it was shown in the present case.

Elucidation of the role of the bile in the esophageal carcinogenesis is complicated by the intrinsic complexity of the esophageal tissue, which is made up of many different, but interacting cell types. Synchronous neoplasm formation with different growth pattern characteristics is a rarity in both humans and model experiments. Interestingly, this suggests that the experimental model presented is applicable for mimicking the human pathology and etiology of the persistent GERD-induced carcinogenesis.

5.3 Hungarian specialties in esophageal columnar metaplasia and carcinogenesis

From the time when Paull classified the columnar metaplasias of the esophagus into cardiac, fundus and intestinal types new aspects had emphasized the clinical behavior of each type, and cardiac and fundus metaplasias are separated from the intestinal one since they have no malignant potential. As far as intestinal metaplasia is concerned, it has also been divided into CIM and SIM. CIM was thought to close to conventional intestinal metaplasia of the stomach with low malignant potential, while the specialized one proved to have high tendency to turn into malignant change recalled alone as BE according to the claim of the National Academy of Gastroenterologists. Thus pathologists and gastroenterologists term it BE if they diagnose SIM. Nevertheless, the British and Japanese definition of BE does not need the identification of SIM. In this human BE study we showed that the pure SIM is very rare in the Hungarian population accounting for 4.1%. Cases containing focal SIM accounted for not more than ¼ of patients, in contrast with the data of United States of America. Since the endoscopic biopsies were performed in one gastroenterological center providing accurate and reproducible endoscopic data, the fact that samples are artificial, is excluded. Although other extensive epidemiological study on BE has not been published so far in Hungary, according to the available data the Hungarian prevalence of EAC is still low and has not been significantly increased as it is seen in the US, or in the Western-European countries. This may explain the relatively low prevalence of SIM in the studied patients. Therefore our results may strengthen the state of another Hungarian workgroup

that esophageal (Barrett-derived) adenocarcinoma is also rare in the Hungarian population comparing to other European countries which needs for further investigations. The low ratio of SIM in the samples points out the role of other main and associated metaplasias in the metaplastic process.

5.4 Non-specialized intestinal counterparts are dominated in metaplastic columnar mucosa

According to detailed assessment of the metaplasias listed above it is important to state that these metaplasias showed more mosaic type of distribution than zonal type described by Paull. The obtained data confirm the SIM-based definition of the BE since SIM is the only type of metaplasia in the esophagus that carries a cancer risk. However, the other types form a spectrum of columnar lined esophagus and, as the authors state, are important to recognize because they provide information regarding genesis of SIM. In our material we showed that 84% of SIM cases contained cardiac mucosa with pseudogoblet cells as well, suggesting the potential role of the cardiac glands in the early propagation of the metaplastic process. Indeed, important data have been shown that cardiac mucosa is not a normal counterpart at the squamocolumnar junction; it occurs due to gastroesophageal reflux. Morphologically, the lucency of cardiac glands is not as great as that of superficial mucous glands (Figure 12) and the contained mucin is frequently sialized or sulphated which can be easily shown by PAS – AB (pH=2.5) and GAF-AB stainings. The latest phenomenon is called ’pseudogoblet cell’ which may testify a transitional state into goblet cell. For the diagnosis of the SIM metaplasia the presence of pseudogoblet cells, in addition of true goblet cells, is also required. The fact that 12 cases with cardiac mucosa displayed low grade dysplasia indicates facultative premalignancy in the case of cardiac glands as well. The fundic mucosa seems so far not to play an important role in the metaplastic process. Although it can be associated with SIM, these glands are fully separated from the intestinal ones. Hence they can be regarded as remnants, which are similar to those located normally in the squamocolumnar junction without any malignant potential. In our material there were no dysplastic fundic glands as well. Pancreatic acinar metaplasia is a particular but not rare finding in the esophagus as well as in the stomach, especially located near to the GEJ. It has been also detected in the cervical inlet patch of esophagus and presence of pancreatic acini has been described in the autoimmune gastritis samples; however, their role is not known. In our material pancreatic acinar metaplasia decreased the risk for the formation of SIM with an odds ratio of 0.45. Co-localization with pure SIM was rare (1/29 cases). However, in 4 cases they coexisted with intestinal metaplasia even with low or high grade dysplasia. Nevertheless, if they are seen without intestinal metaplasia, dysplasia was not diagnosed. Thus presence of the pancreatic acinar metaplasia (without intestinal metaplasia) carries less severe pathologic process. Respiratory metaplasia contains ciliated pseudostratified mucosa, usually found near MLE consisting of both squamous and mucous cells. In our material it was a rare finding (accounted for 0.2%) but they coexisted with the cardiac pseudogoblet cells and one of them showed direct transition into SIM as published in a Japanese study.

5.4.1 One more possible origin of metaplastic mucosa – introducing superficial mucous glands

It is about 130 years since Rüdinger first described the tubulo-acinous glands of the lower esophagus lying above the muscularis mucosae. They were distinguished from glands located in the submucosa. Rüdinger's works were unnoticed until Schaffer called attention to them in 1897. Then these mucinous glands remained again neglected; thus they are usually misinterpreted as cardiac glands. In our material superficial mucous glands coexisted with all main metaplasias, but they showed the most frequent association with the cardiac and intestinal metaplasia. Furthermore, within superficial mucous glands we have often identified pancreatic acinar cell metaplasia, and cardiac gland transition and direct SIM transition. Although it is not the only source of the columnar metaplasia in the experimental GERD carcinogenesis, the formation of metaplasia in superficial mucous glands is likely present. Indeed, CDX2-immunostained picture provide histopathological evidence, how this type of metaplasia forms. Moreover, superficial mucous glands are phenotypically completely reminiscent to ulcer associated cell lineage which is a well established histopathological entity in the regeneration of the foregut and hindgut epithelium. Some superficial mucous glands are usually present in the squamocolumnar junction, but in case of reflux damage they are increasing in number and appearing to upper direction in the metaplastic area. The presence of superficial mucous glands in the gastric cardia can be responsible for controversies about the existence of cardiac glands in normal condition since superficial mucous glands can be misdiagnosed with them. Some superficial mucous glands are usually present in the squamocolumnar junction, but in case of reflux damage they are increasing in number and appearing upper direction in the metaplastic area. Nevertheless, the superficial mucous glands should be distinguished from cardiac glands based on their routine histomorphology.

5.5 Complicating BE

As we showed underneath the pathogenesis of the esophageal columnar metaplasia is more complex than is typically calculated from the current definition which, therefore, should be reviewed. Due to reflux damage superficial mucous glands can be served to compose foregut-derived tissues as cardiac, fundic, pancreas acinar and respiratory metaplasia. SIM also can be formed by direct shift from the superficial mucous glands and respiratory metaplasia as well as from the cardiac mucinous cells through pseudogoblet cell change. The latter is responsible for the vast majority of the cases.

The findings listed suggest that the histopathological report should contain, as our histopathological dataset showed, all of the main and additional metaplasias found in the sample, independently of the presence or absence of SIM. Only detailed and comparative histopathological view will create the field for further clinicopathological considerations and refinements of BE.

5.6 EMR is necessary for not just treatment but for accurate diagnosis of preneoplastic and early neoplastic lesions in the foregut

The first indications of EMR mainly were curative for early cancers but certain cases are palliative for those who are not fit for operation replacing surgery. As well as the diagnostical value of EMR considered the endoscopic mucosal biopsies obtained with standard biopsy forceps can give false-negative results, especially if the epithelial layer is not involved in the pathological process. In contrast, EMR involves *en bloc* resection of the entire lesion, histological examination of which is clearly more reliable than the forceps biopsy, which may not be representative of the entire lesion. The complete agreement between the histological results on the forceps biopsy samples and the resected specimens in 76.7% of the cases, and the sensitivity and specificity of forceps biopsy for the diagnosis of neoplastic lesions 87.5% and 65.2%, respectively, appear relatively satisfactory. However, the diagnosis based upon the histological results of the forceps biopsy samples may be a misdiagnosis. Indeed, flat lesions would have been misdiagnosed from only the histology of the forceps biopsy sample. The biopsy specimens do not represent the whole lesion: the material may be insufficient to establish a correct diagnosis, or focal cancers may be missed. It is therefore recommended to remove flat lesions at the time of endoscopy in order to establish the final diagnosis after a thorough histological examination of the entire lesion. We diagnosed seven cases of EGC in the 6-year study period, and complete agreement between the histological results on the forceps biopsy and the resected specimens was found in only 28.5% of these lesions. In three cases, foci of well-differentiated intramucosal carcinoma were present in the resected specimens, but were missed on the biopsy sampling. This is not highly relevant clinically as all adenomas must be completely removed, because of the adenoma-carcinoma sequence. Since the lack of recurrence and serious complications EMR of all flat lesions larger than 5 mm should be considered not just for therapeutic but for diagnostical purposes. EMR has been also successfully introduced to treat BE related intraepithelial neoplasias being effective treatment modality. Comparing to surgery, EMR associated with a lower morbidity rate and decreased the risk for procedure-related mortality. However, the recurrence rate is higher in patients treated with EMR, therefore follow-up procedures are mandatory.

PUBLICATIONS DIRECTLY RELATED TO THE SUBJECT OF THE DISSERTATION

I. ***Carcinogenetic pathways in experimental gastroesophageal reflux:***

Szentpáli K, Széll M, Paszt A, Wolfárd A, Dobozy A, **Németh I**, Tiszlavicz L, Iván L, Boros M: Simultaneous adeno- and squamous cell carcinoma with different phenotypic profiles in a rat model of chronic gastroesophageal reflux. Dis Esophagus. 2007; 20: 305-10.

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II. ***Histopathologic relations in routine diagnostics of the foregut:***

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