

## Preneoplastic Processes in the Esophagus

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In some areas particularly the United States the incidence of esophageal adenocarcinoma has dramatically increased in the last two decades while the incidence of esophageal squamous cell carcinoma has decreased in certain societies. In short, esophageal adenocarcinoma is neoplasm which is the result of repetitive injury related to reflux disease; its progression follows through the stages of metaplasia, dysplasia, and invasion (carcinoma). While squamous cell carcinoma as well follows a dysplasia carcinoma sequence and has a pathogenesis related to prior epithelial injury, the injurious factors differ; smoking, alcohol use, and/or caustic injury due to lye ingestion are key factors in esophageal squamous cell development. The role of human papilloma virus infection is controversial. Reflux disease, while a form of caustic injury, is not a causative factor in esophageal squamous cell carcinoma. As well, there is no metaplasia in this neoplastic pathway.

Adenocarcinoma and squamous carcinoma of the esophagus are two distinct entities with divergent epidemiology, risk factors, genetics, and phenotypes. The recent increase in adenocarcinoma is most likely related to a multitude of factors including genetics, environment, and likely life style. Specifically, the Western diet and obesity epidemic has paralleled the increased incidence of esophageal adenocarcinoma. Additionally, in the Western type societies, the advent of the recognition of *H. pylori* and its association with peptic ulcer disease has led to antibiotic treatment; thus, while *H. pylori* gastritis may be decreasing in these areas, the incidence of esophageal adenocarcinoma has increased.

The association of esophageal adenocarcinoma with intestinal metaplasia is at this time undeniable. Genetic and/or epigenetic changes are important in both pathways of neoplastic progression. Despite the seeming paucity of true knowledge with regard to the molecular biology of the process, the pathogenesis of esophageal adenocarcinoma is one fundamentally related to reflux. Repeated injury to the squamous lined esophagus with not only gastric contents but importantly duodenal contents which include bile acid may drive metaplasia of the squamous epithelium to glandular epithelium containing in part goblet cells (intestinal metaplasia). However, it is not clear why only a minority of individuals with reflux develop metaplasia. While obesity is thought to increase the risk of reflux disease, a meta-analysis by Cook showed that increased BMI had no correlation with risk to progression to Barrett esophagus.

There are multiple control elements which maintain and signal cells for lineage differentiation. Caudal-related homeobox 2 (*Cdx2*) is a transcription factor which controls cellular differentiation toward the intestinal phenotype. Transgenic animal models in which *Cdx2* has been expressed in the stomach and/or esophagus develop intestinal epithelium in the targeted areas. Silberg developed a transgenic mouse and showed that *Cdx2* expression alone was sufficient to give an intestinal phenotype. Because of this critical role of *Cdx2*, many studies have focused on understanding the mechanisms of control of this transcription factor and its potential use as a diagnostic marker of Barrett's esophagus.

As *Cdx2* is a key transcription factor for intestinal differentiation, one may ask the question as to what keeps the esophageal squamous epithelium in the differentiated squamous state. Because expression of *Cdx2* is sufficient to cause intestinal differentiation, one may

therefore hypothesize that Cdx2 is not expressed in esophageal squamous epithelium. Indeed, Cdx2 is not expressed in these cells. The control of gene expression is complex involving chromatin configuration, histone acetylation and methylation of promoter sequences. Genes with methylation of cytosine bases in the promoter region are silenced. Thus, promoter methylation is one control mechanism of gene expression. Using squamous esophageal cell lines, Liu showed that exposure to acid and/or bile acids may activate Cdx2 expression in human esophageal epithelial cells through promoter demethylation. As a corollary, in squamous cell esophageal carcinoma, Cdx2 is silenced by continued methylation of its promoter (Guo) highlighting the importance of epigenetic mechanisms in neoplastic progression.

Several controversial issues exist around the exact definition of Barrett esophagus particularly in relationship to “cardiac” and gastroesophageal junction adenocarcinomas. To understand these controversies, we must first realize that the importance of identifying patients as having “Barrett’s esophagus” is to identify those patients with an increased risk of progressing to esophageal adenocarcinoma. Barrett in 1950 first observed columnar epithelium in the esophagus and interpreted this finding as a congenitally shortened esophagus creating a mediastinal stomach. Three years following this initial observation, Allison and Johnstone refined Barrett’s initial hypothesis and showed that the true esophagus was lined by glandular epithelium (“gastric membrane”) and suggested not only that this epithelium was acquired but also postulated reflux as an etiology. The malignant potential of this glandular epithelial lining the esophagus and its relationship to esophageal adenocarcinoma eventually became clear in the 1970s. Further studies followed which implicated special intestinal type mucosa as being the pre-neoplastic culprit.

Thus, we currently believe that intestinal metaplasia of the esophagus is acquired through mostly asymptomatic reflux disease, and places the patient at increased risk of developing adenocarcinoma compared to patients not having Barrett esophagus. We also currently believe that once acquired, this epithelium may undergo evolution to dysplasia and further evolution to carcinoma through a series of genetic mutations.

When examining a biopsy from a patient who has undergone upper endoscopy, several questions may arise when considering a diagnosis of Barrett esophagus. Currently, gastric type mucosa (foveolar) is not universally considered to have the neoplastic progressive potential as intestinal type epithelium and is not in itself in an adult considered “Barrett’s” by some. The data for this statement derives from observations in the pediatric population where foveolar type gastric mucosa may be found > 3 cm. above the GE junction. These patients virtually never progress to adenocarcinoma. In addition, the gastric mucosa over time regresses in this population.

The finding of fundic gastric mucosa in a presumed esophageal biopsy should incite several questions and in no way be considered Barrett type metaplasia. The most common clinical scenario yielding this biopsy is a hiatal hernia. A portion of the stomach slides above the diaphragm yielding a false impression of the location of the GE junction. As the lower GE sphincter is purely functional and not a structural entity, one can readily understand how the endoscopist may interpret their biopsy location as esophageal. Often when asked, the clinician indeed does know that the patient has a hernia.

There are two additional rare situations, which may yield similar biopsy findings. The first is the inlet patch. Inlet patches are islands of gastric epithelium usually about 1-2 cm. in diameter, which occur, in the upper esophagus. This heterotopic mucosa has virtually no neoplastic potential although rare cases of adenocarcinomas have been reported. Discussion

with the clinician as to the location and the appearance of the surrounding epithelium will clarify the diagnosis. The second rare situation is an esophageal duplication cyst. These congenital abnormalities are out pouchings of the esophagus which may be lined by a variety of epithelial types including gastric fundic, foveolar, respiratory, and squamous. Thus, one needs to ask about the radiologic and endoscopic appearance of the biopsied area. While management of duplications cysts is controversial, adenocarcinomas have arisen in these areas although rarely.

The finding of intestinal metaplasia in the GE junction and cardia raises several issues but currently, regardless of the nomenclature, is considered akin to Barrett esophagus or so-called "short-segment Barrett's." Specifically, "Barrett's esophagus" originally was considered to be glandular metaplasia which extended >3 cm. above the GE junction giving rise to the prototypical pink salmon tongue endoscopic appearance. As previously stated, we have further refined the definition to require intestinal metaplasia and do not require a "minimum height" above the GE junction. Recently, the concept of "short-segment Barrett's esophagus" has emerged particularly in conjunction with cardia/GE junction adenocarcinomas.

Cameron et al (1995) examined GE junction tumors to assess the frequency of their association with intestinal metaplasia in the adjacent mucosa. They found the majority of cases to indeed have associated intestinal metaplasia. Thus, these tumors are believed to have arisen in a manner similar to adenocarcinomas in prototypical Barrett's -i.e., reflux-intestinal metaplasia-dysplasia-carcinoma. In this study, they defined an adenocarcinoma as being esophageal, junctional, and gastric if the midpoint of the tumor was >2 above, ≤2cm below, and >2 below the GE junction, respectively. They considered Barrett type epithelium to be present in GE junction tumors if intestinal metaplasia was found proximal to the tumor. They found intestinal metaplasia (Barrett's) in 67% of tumors ≤6cm in length. In 50% of cases showing intestinal metaplasia, the length of Barrett's was less than 3cm. Additionally, intestinal metaplasia was often found in the cardia.

Spechler et al (1994) have taken a slightly different tack. The goal of their study was to determine the incidence of intestinal metaplasia at the GE junction without associated endoscopically visible "Barrett's" (i.e., "short-segment Barrett's") in patients without adenocarcinoma. The patients who underwent endoscopy were referred mostly for workup of heartburn, pain, dyspepsia, and dysphasia. Of the patients with no endoscopically apparent "Barrett's", biopsies of the GE junction showed intestinal metaplasia in 18% of patients.

Locke et al (1995) have tried to further clarify the controversy often surrounding the nomenclature for adenocarcinoma of the GE junction/cardia. The concept that GE junction carcinomas may be distinct from gastric tumors has only recently been accepted. Prior epidemiologic classifications have placed GE junction tumors together with gastric cardiac tumors in the Intestinal Classification of Diseases for Oncology. Epidemiologic studies have shown that the incidence of distal gastric adenocarcinoma has decreased over time in this country. Locke et al hypothesized and proved that the reported increase in cardiac carcinomas is in fact an increase in GE junction tumors and not true cardiac tumors.

The debate of the method of classifying cardiac carcinomas will undoubtedly continue as etiologic factors and precursor lesions are determined. Hansson et al (1993 and 1995) found that "cardiac" adenocarcinomas were not associated with seropositivity for H. pylori while antral and body gastric carcinomas were highly associated. Additionally, they found equal prevalence of H. pylori positivity among the diffuse and intestinal forms of gastric carcinoma. Whether their definition of cardiac tumors could as well be classified as GE junction tumors as per the study of Locke et al is not known.

Functionally, with the emergence of “short segment Barrett’s”, clinicians are no longer with confidence be able to exclude the presence of intestinal epithelium solely by endoscopic appearance and biopsies of the GE junction should be obtained regardless of the endoscopic appearance. “Short segment Barrett’s” esophagus exists and is associated with adenocarcinomas of the GE junction.

Even more problematic is evaluating a polyp near the GE junction. Histologically the polyp is inflammatory/hyperplastic with foci of intestinal metaplasia. Should this finding be considered “short segment Barrett’s” or is this intestinal metaplasia in a hyperplastic gastric polyp?

By some current definitions, the epithelium must be at the surface to fulfill the definitional criteria. One must of course assure that the biopsy is not tangential leading to a misinterpretation. Sampliner et al (1988) in a study of 45 patients with Barrett esophagus found squamous epithelium overlying intestinal type epithelium in seven patients, none of whom had anti-reflux surgery. This situation has also arisen in studies of laser therapy for Barrett type epithelium. Laser phototherapy is a modality aimed at eradicating the intestinal type epithelium. Obliteration of this type with re-epithelialization by squamous epithelium is thought to eradicate the risk of neoplastic progression. Although the overall therapeutic outcome has yet to be fully determined, on post-laser biopsy, the surface epithelium was indeed squamous but the submucosal area demonstrated intestinal type glands.

Once a patient is identified as having Barrett metaplasia, he/she should undergo regular endoscopies with biopsies to determine the presence of dysplasia. However, the cost effectiveness of this approach is questioned. The most probable time of finding a significant neoplastic lesion is at the time of first endoscopy. Unfortunately, as previous studies have shown, many people with Barrett esophagus are asymptomatic and may present with carcinoma. We, of course, wish to identify and follow these patients before they progress to carcinoma. How to evaluate these patients and what criteria to follow in managing patients is a subject of much study. The histological definition and grading of dysplasia in Barrett esophagus is the same scheme used in IBD with associated inter and intraobserver variability. As with Riddell’s study for IBD, Reid et al found a good concordance of opinions with high-grade dysplasia among experienced GI pathologists. However, the level of variability was considerable for low-grade dysplasia and reactive atypia. Thus, dysplasia when present should be graded as indefinite, low or high. Additionally, the accepted management of a patient with low grade and indefinite for dysplasia is close follow up. Controversy arises when a high-grade dysplasia diagnosis is made. Reid et al (1988) have shown that high-grade dysplasia can be found in Barrett’s mucosa without a grossly visible lesion. While esophagectomy for a diagnosis of carcinoma is one option, the surgical management of high-grade dysplasia has drawn debate as the natural history of this lesion alone is not known. It is estimated that progression from low-grade dysplasia to high-grade dysplasia may take approximately two years. Progression from high-grade dysplasia to cancer may take about one year, although by five years 50% of patients may develop carcinoma. In light of the fact that not everyone with high grade dysplasia develops carcinoma by 5-years, Reid et al (1993) have proposed a rigorous endoscopic surveillance program in an attempt to follow patients with high-grade dysplasia to detect the evolution to surgically treatable carcinoma. Similarly, Burgess et al found that the *extent* of high grade dysplasia in mucosal biopsies is related to prognosis. Specifically, they found that patients with focal high grade dysplasia defined as a cluster of 5 or less high grade dysplastic glands had a better long term outcome than those with more extensive high grade dysplasia. In the Hines VA study (Schnell et

al), only 16% of patients with high grade dysplasia progressed to cancer in 14 years. The efficiency of this surveillance protocol awaits further study.

As with IBD, ancillary tests to histology have been sought. Raskind et al (1992) have shown that Barrett type epithelium may contain clonal areas of cells with cytologic abnormalities which may progress over time to high-grade dysplasia and carcinoma. Yoreves et al (1993) and Ramel et al (1992) have shown p53 accumulation in Barrett type epithelium correlates with dysplasia. However, they also detect p53 accumulation in morphologically nondysplastic epithelium and suggest that alteration in p53 may be an early event in neoplastic progression in Barrett type metaplasia. Additionally, neoplastic progression in Barrett esophagus has been associated with multiple cell cycle abnormalities. Additional tests such as immunohistochemical staining for alpha-methylacyl-Coa-racemase have gained attention as another potential biomarker to help distinguish dysplastic from reactive epithelial cells.

In summary, Barrett esophagus is an acquired metaplastic process, which may be defined as intestinal metaplasia (i.e. true goblet cell formation) at or above the GE junction; there is controversy regarding the neoplastic potential of non-intestinal type epithelium in this area. Endoscopy is not sufficient to exclude Barrett's metaplasia with the identification of short-segment Barrett's and its association with GE junction adenocarcinomas. The management of patients with high grade dysphasia is somewhat controversial but at the very least confirmation of the histologic findings by a second opinion may be useful. Esophagectomy as a therapeutic option in recently instituted high grade dysplasia is one option; however, the utility of surveillance protocols for high grade dysplasia and the role of endoscopic mucosal resections await further evaluation.

The epidemiology and molecular pathogenesis of squamous cell carcinoma differs dramatically compared to that of adenocarcinoma. Squamous cell carcinoma is the most common primary esophageal malignancy. While the dysplasia carcinoma sequence is in play, clearly there is no role for metaplasia. Smoking, alcohol use, achalasia, and/or prior lye ingestion are key pathogenic factors. Infection with high risk human papilloma viruses (HPV) is a central mechanism in cervical squamous neoplastic progression. The role of high risk HPV infection in the pathogenesis of esophageal squamous cell carcinoma is debated. While several studies found no high risk HPV DNA in squamous cell carcinomas in Korean and Chinese populations, another study examining a population from Chile found that 29% of these cancers harbored HPV.

From a molecular pathogenesis stand point, esophageal squamous cell carcinomas have a high degree of chromosomal instability with gross chromosomal aberrations including bridges. The mechanism of this instability may be multifactorial. Kammor has shown that the telomere length in normal appearing squamous epithelium adjacent to an esophageal squamous cell carcinoma is shorter than normal. This shortening may predispose these phenotypically normal cells to undergo chromosomal modifications which may lead to cancer. This shortening may reflect the result of continued injury with cell turnover and proliferation. Using a transcriptome type of analysis with SAGE, van Baal found that BMP4, E-Cadherin and TFF3 were the most up-regulated genes in esophageal squamous cell carcinomas compared to normal squamous epithelium. Claudins are a family of molecules important for tight junction formation. Disruption of these molecules has been implicated not only in inflammatory bowel disease but also neoplastic progression in the squamous esophagus. Decreased expression of claudin 7 is found in esophageal squamous cell carcinomas. In cell lines, invasive ability increases with its down

regulation. Additionally, claudin 7 may interact with E-cadherin, another cell adhesion molecule; specifically, loss of claudin 7 is associated with decreased expression of E-cadherin (Lioni). The retinoblastoma (Rb) pathway is important in cell cycle control. Progressive loss of its function as shown by lack of expression of pRb occurs with increasing grades of squamous dysplasia in the esophagus (Contu). Cyclooxygenase-2 (COX-2) was also found to have progressively increased expression with grades of dysplasia (Zhi); this finding gives credence to several chemo-preventive trails using non-steroidal anti-inflammatory agents.

Dysplasia, once thought to be either static or progressive, in actuality may have the ability to regress. Hence, chemo-preventive trails have been initiated with the goal of either halting further progression or potentially reversing the dysplastic field. These chemo-preventive trails have met with varying success. A trail examining selenomethionine and celecoxib ability to effect the natural history of squamous cell carcinoma (Joshi) showed that some patients progressed and some patients regressed with regard to their dysplasia. Those patients who regressed had by gene analysis higher levels of expression of those genes involved in immune stimulation; the opposite was true for those who progressed. Thus, the immune system may play a critical role in modulating neoplastic progression in the esophagus.

As with adenocarcinoma, early detection of dysplastic lesions is important to prevent further neoplastic progression. Confocal laser endomicroscopy may be one modality to accurately detect such preneoplastic lesions. As with Barrett's, endomucosal resection (EMR) is one form of therapy. EMR may be curative in both situations even with invasion into the muscularis mucosa (Katada).

Thus, while adenocarcinoma and squamous cell carcinoma both share a dysplasia carcinoma sequence, they are very separate entities in terms of epidemiology and molecular pathogenesis. As with many neoplastic processes, they share features of early dysregulation of cell cycle check points and chromosomal instability. Additionally, both processes may share epigenetic modifications as part of neoplastic development.

1. Ahmad NA, Kochman ML, Long WB, Furth EE, Ginsberg GG. Efficacy, safety and clinical outcomes of endoscopic mucosal resection: a study of 101 cases. *Gastrointestinal Endoscopy* 55(3):390-6, March 2002.
2. Amano Y. Kushiya Y. Yuki T. Takahashi Y. Chinuki D. Ishimura N. Furuta K. Ishihara S. Adachi K. Maruyama R. Kinoshita Y. Predictors for squamous re-epithelialization of Barrett's esophagus after endoscopic biopsy. *Journal of Gastroenterology & Hepatology*. 22(6):901-7, 2007 Jun
3. American Society for Gastrointestinal Endoscopy: The role of endoscopy in the surveillance of premalignant conditions of the upper gastrointestinal tract. Guidelines for clinical application. *Gastrointest Endosc* 34:18S-20S, 1988.
4. Burgess JN, Payne WS, Andersen HA, Weiland LH and Carlson HC: Barrett esophagus. The columnar-epithelial-lined lower esophagus. *Mayo Clin Proc* 46:728-734, 1971.
5. Buttar NS. Wang KK. Sebo TJ. Riehle DM. Krishnadath KK. Lutzke LS. Anderson MA. Petterson TM. Burgart LJ. Extent of high-grade dysplasia in Barrett's esophagus correlates with risk of adenocarcinoma. *Gastroenterology*. 120(7):1630-9, 2001
6. Cameron AJ, Lomboy CT, Pera M and Carpenter HA: Adenocarcinoma of the esophagogastric junction and Barrett's esophagus. *Gastroenterology* 109:1541-1546, 1995.

7. Cameron AJ, Zinsmeister AR, Ballard DJ and Carney JA: Prevalence of columnar-lined (Barrett's) esophagus. *Gastroenterology* 99:918-922, 1990.
8. Caplan MS, Sun XM and Hsueh W: Hypoxia causes ischemic bowel necrosis in rats: The role of platelet-activating factor (PAF-Acether). *Gastroenterology* 99:979-986, 1990.
9. Castillo A., Aguayo F., Koriyama C., Torres M., Carrascal E., Corvalan A, Roblero JP., Naquira C., Palma M., Backhouse C., Argandona J., Itoh T., Shuyama K., Eizuru Y., Akiba S. "Human papillomavirus in esophageal squamous cell carcinoma in Colombia and Chile." *World Journal of Gastroenterology*. 12(38):6188-92, 2006 Oct 14.
10. Chen X. Qin R. Liu B. Ma Y. Su Y. Yang CS. Glickman JN. Odze RD. Shaheen NJ. "Multilayered epithelium in a rat model and human Barrett's esophagus: similar expression patterns of transcription factors and differentiation markers." *BMC Gastroenterology*. 8:1, 2008.
11. Collen MJ, Lewis JH and Benjamin SB: Gastric acid hypersecretion in refractory gastroesophageal reflux disease. *Gastroenterology* 98:654-661, 1990.
12. Contu, S.S., Contu, P.C., Damin, D.C., Fagundes, R.B., Bevilacqua, F., Rosa, A.S., Prolla, J.C., Moreira, L.F. "pRB expression in esophageal mucosa of individuals at high risk for squamous cell carcinoma of the esophagus." *World Journal of Gastroenterology*. 13(11):1728-31, 2007 Mar 21.
13. Cook, M. B., Greenwood, D., Hardie, C, Wild, L. J., C. P., Forman, D. A systematic review and meta-analysis of the risk of increasing adiposity on Barrett's esophagus. *American Journal of Gastroenterology*. 103(2):292-300, 2008 Feb.
14. Dahms BB and Rothstein FC: Barrett's esophagus in children: A consequence of colonic gastroesophageal reflux. *Gastroenterology* 86:318-323, 1984.
15. Das KM, Prasad I, Garia S and Amenta PS: Detection of a shared colon epithelial epitope on Barrett epithelium by a novel monoclonal antibody. *Ann Intern Med* 120:753-756, 1994.
16. Garside, R. Pitt, M. Somerville, M. Stein, K. Price, A. Gilbert, N. "Surveillance of Barrett's esophagus: exploring the uncertainty through systematic review, expert workshop and economic modelling." *Health Technology Assessment (Winchester, England)*. 10(8):1-142, iii-iv, 2006 Mar
17. Gray MR, Hall PA, Nash J, Ansari B, Lane DP and Kingsnorth AN: Epithelial proliferation in Barrett's esophagus by proliferating cell nuclear antigen immunolocalization. *Gastroenterology* 103:1769-1776, 1992.
18. Guo, MZ, House, M. G., Suzuki, H., Ye, Y., Brock, M.V., Lu, F., Liu, Z., Rustgi, A.K., Herman, J.G. "Epigenetic silencing of CDX2 is a feature of squamous esophageal cancer." *International Journal of Cancer*. 121(6):1219-26, 2007 Sep 15.
19. Haggitt RC: Barrett's esophagus, dysplasia, and adenocarcinoma. *Hum Pathol* 25:982-993, 1994.
20. Hamelin R, Fléjou JF, Muzeau F, Potet F, Laurent-Puig P, Fékété F and Thomas G: TP53 gene mutations and p53 protein immunoreactivity in malignant and premalignant Barrett's esophagus. *Gastroenterology* 107:1012-1018, 1994.
21. Hamilton SR and Yardley JH: Regeneration of cardiac type mucosa and acquisition of Barrett mucosa after esophagogastrectomy. *Gastroenterology* 72(4):669-675, 1977.
22. Hansson LE, Engstrand L, Nyrén O, Evans DJ, Lindgren A, Bergström R, Andersson B, Athlin L, Bendtsen O and Tracz P: *Helicobacter pylori* infection: Independent risk indicator of gastric adenocarcinoma. *Gastroenterology* 105:1098-1103, 1993.

23. Hu, Y., Williams, V. A. Gellersen, O., Jones, C. Watson, T. J. Peters, J. H. "The pathogenesis of Barrett's esophagus: secondary bile acids upregulate intestinal differentiation factor CDX2 expression in esophageal cells." *Journal of Gastrointestinal Surgery*. 11(7):827-34, 2007 Jul.
24. Jass JR: Mucin histochemistry of the columnar epithelium of the oesophagus: a retrospective study. *J Clin Pathol* 34:866-870, 1981.
25. Joshi, N., Johnson, L., L., Wei, W.Q., Abnet, C.C., Dong, Z.W., Taylor, P.R., Limburg, P.J., Dawsey, S. M., Hawk, E.T., Qiao, Y.L., Kirsch, I.R. "Gene expression differences in normal esophageal mucosa associated with regression and progression of mild and moderate squamous dysplasia in a high-risk Chinese population." *Cancer Research*. 66(13):6851-60, 2006 Jul.
26. Kammori, Makoto. Poon, Steven S S. Nakamura, Ken-Ichi. Izumiyama, Naotaka. Ishikawa, Naoshi. Kobayashi, Masahiko. Naomoto, Yoshio. Takubo, Kaiyo. Squamous cell carcinomas of the esophagus arise from a telomere-shortened epithelial field. *International Journal of Molecular Medicine*. 20(6):793-9, 2007 Dec.
27. Katada, C. Muto, M. Momma, K. Arima, M. Tajiri, H. Kanamaru, C. Ooyanagi, H. Endo, H. Michida, T. Hasuike, N. Oda, I. Fujii, T. Saito, D. "Clinical outcome after endoscopic mucosal resection for esophageal squamous cell carcinoma invading the muscularis mucosae--a multicenter retrospective cohort study. *Endoscopy*. 39(9):779-83, 2007 Sep.
28. Koh JS., Lee SS., Baek HJ., Kim YI. "No association of high-risk human papillomavirus with esophageal squamous cell carcinomas among Koreans, as determined by polymerase chain reaction." [Journal Article] *Diseases of the Esophagus*. 21(2):114-7, 2008. UI: 18269645
29. Lee E, Schiller LR, Vendrell D, Santa Ana CA and Fordtran JS: Subepithelial collagen table thickness in colon specimens from patients with microscopic colitis and collagenous colitis. *Gastroenterology* 103:1790-1796, 1992.
30. Levine DS, Haggitt RC, Blount PL, Rabinovitch PS, Rusch VW and Reid BJ: An endoscopic biopsy protocol can differentiate high-grade dysplasia from early adenocarcinoma in Barrett's esophagus. *Gastroenterology* 105:40-50, 1993.
31. Lioni, M., Brafford, P., Andl, C., Rustgi, A., El-Deiry, W., Herlyn, M. "Dysregulation of claudin-7 leads to loss of E-cadherin expression and the increased invasion of esophageal squamous cell carcinoma cells." *American Journal of Pathology*. 170(2):709-21, 2007 Feb.
32. Locke GR, Talley NJ, Carpenter HA, Harmsen WS, Zinsmeister AR and Melton LJ: Changes in the site- and histology-specific incidence of gastric cancer during a 50-year period. *Gastroenterology* 109:1750-1756, 1995.
33. Madding GF, Baer LS and Kennedy PA: Gastric syphilis. A case report. *Ann of Surgery* 159(2):271-274, 1964.
34. Montgomery et al: Dysplasia as a Predictive Marker for Invasive Carcinoma in Barrett Esophagus: A Follow-up Study Based on 138 Cases From a Diagnostic Variability Study. *Hum Pathol* 32:379388, 2001.
35. Paull A, Trier JS, Dalton D, Camp RC, Loeb P and Goyal RK: The Histologic spectrum of Barrett's esophagus. *N Engl J Med* 295:476-480, 1976.
36. Pera M., Pera M., de Bolos C. Brito M.J., Palacin A., Grande L., Cardesa A., Poulson R. "Duodenal-content reflux into the esophagus leads to expression of Cdx2 and Muc2 in areas of squamous epithelium in rats." *Journal of Gastrointestinal Surgery*. 11(7):869-74, 2007 Jul.

37. Ramel S, Reid BJ, Sanchez CA, Blount PL, Levine DS, Neshat K, Haggitt RC, Dean PJ, Thor K and Rabinovitch PS: Evaluation of p53 protein expression in Barrett's esophagus by two-parameter flow cytometry. *Gastroenterology* 102:1220-1228, 1992.
38. Raskind WH, Norwood T, Levine DS, Haggitt RC, Rabinovitch PS and Reid BJ: Persistent clonal areas and clonal expansion in Barrett's esophagus. *Cancer Research* 52:2946-2950, 1992.
39. Reid BJ, Haggitt RC, Rubin CE, Roth G, Surawicz CM, Van Belle G, Lewin K, Weinstein WM, Antonioli DA, Goldman H, MacDonald W and Owen D: Observer variation in the diagnosis of dysplasia in Barrett's esophagus. *Hum Pathol* 19:166-178, 1988.
40. Reid BJ, Sanchez CA, Blount PL and Levine DS: Barrett's esophagus: Cell cycle abnormalities in advancing stages of neoplastic progression. *Gastroenterology* 105:119-129, 1993.
41. Reid BJ, Weinstein WM, Lewin KJ, Haggitt RC, VanDeventer G, DenBesten L and Rubin CE: Endoscopic biopsy can detect high-grade dysplasia or early adenocarcinoma in Barrett's esophagus without grossly recognizable neoplastic lesions. *Gastroenterology* 94:81-90, 1988.
42. Sampliner RE, Steinbronn K, Garewal HS and Riddell RH: Squamous mucosa overlying columnar epithelium in Barrett's esophagus in the absence of anti-reflux surgery. *Am J Gastro* 83(5):510-512.
43. Schnell TG, Sontag SJ, Chejfec G, Aranha G, Metz A, O'Connell S, Seidel UJ, Sonnenberg A. Long-term nonsurgical management of Barrett's esophagus with high-grade dysplasia. *Gastroenterology*. 120(7):1607-19, 2001 Jun.
44. Shi X, Ying Bhagwandeem, Brahm. Leong, A. "S-Y. CDX2 and villin are useful markers of intestinal metaplasia in the diagnosis of Barrett esophagus." *American Journal of Clinical Pathology*. 129(4):571-7, 2008 Apr.
45. Silberg, D.G., Sullivan, J., Kang, E., Swain, G. P., Moffett, J. Sund, N.J. Sackett, S.D., Kaestner, K. "Cdx2 ectopic expression induces gastric intestinal metaplasia in transgenic mice." *Gastroenterology*. 122(3):689-96, 2002 Mar.
46. Skacel, M., R.E. Petras, et al. (2000). "The diagnosis of low-grade dysplasia in Barrett's esophagus and its implications for disease progression." *The American Journal of Gastroenterology* 95(12):3383-3387.
47. Skinner DB, Walther BC, Little AG: Surgical treatment of Barrett's esophagus. In: Spechler SJ, Goyal R, eds. *Barrett's esophagus: pathophysiology, diagnosis, and management*. New York: Elsevier, 211-221, 1985.
48. Smith RRL, Boitnott JK, Hamilton ST and Rogers EL: The spectrum of carcinoma arising in Barrett's esophagus. A clinicopathologic study of 26 patients. *Am J Surg Pathol* 8:563-573, 1984.
49. Spechler SJ, Sperber H, Doos WG and Schimmel EM: The prevalence of Barrett's esophagus in patients with chronic peptic esophageal strictures. *Digestive Diseases and Sciences* 28(9):769-774, 1983.
50. Spechler SJ, Zeroogian JM, Antonioli DA, Wang HH and Goyal RK: Prevalence of metaplasia at the gastro-oesophageal junction. *Lancet* 344:1533-1536, 1994.
51. Starnes VA, Adkins RB, Ballinger JF and Sawyers JL: Barrett's esophagus. A surgical entity. *Arch Surg* 119:563-567, 1984.

52. Wang, K.K. and R.E. Sampliner (2008). "Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus." *The American Journal of Gastroenterology* 103(3):788-797.
53. van Baal J.W., Bozikas A., Pronk R., Ten K., F.J.W, Milano F. Rygiel A.M., Rosmolen W.D., Peppelenbosch M.P., Bergman J.J., Krishnadath KK. "Cytokeratin and CDX-2 expression in Barrett's esophagus." *Scandinavian Journal of Gastroenterology*. 43(2):132-40, 2008.
54. Wu GD, Beer DG, Moore JH, Orringer MB, Appelman HD and Traber PG: Sucrase-Isomaltase gene expression in Barrett's esophagus and adenocarcinoma. *Gastroenterology* 105:837-844, 1993.
55. Younes M, Lebovitz RM, Lechago LV and Lechago J: p53 protein accumulation in Barrett's metaplasia, dysplasia, and carcinoma: A follow-up study. *Gastroenterology* 105:1637.
56. Zhi, Huiying. Wang, Lin. Zhang, Jian. Zhou, Chuannong. Ding, Fang. Luo, Aiping. Wu, Min. Zhan, Qimin. Liu, Zhihua. "Significance of COX-2 expression in human esophageal squamous cell carcinoma. *Carcinogenesis*. 27(6):1214-21, 2006 Jun.