symposium article
doi:10.1093/annonc/mdm237

Aurora-A overexpression as an early marker of reflux-related columnar mucosa and Barrett's oesophagus

V. Agnese[1†], D. Cabibi[2†], D. Calcara[1], M. Terrasi[1], G. Pantuso[3], E. Fiorentino[3], C. Intrivici[1], G. Colucci[4], F. Aragona[2], N. Gebbia[1], V. Bazan[1] & A. Russo[1*]

[1]Section of Medical Oncology, Department of Surgery and Oncology; [2]Department of Histopathology; [3]Section of Surgical Oncology, Department of Surgery and Oncology, Università di Palermo, Palermo; [4]Division of Medical Oncology, National Institute of Oncology, Bari, Italy

Background: The development of oesophageal adenocarcinoma is generally closely associated with the presence of a specialised intestinal-type epithelium such as that found in Barrett's oesophagus (BO). A particular histological condition is when the distal oesophagus showing cardiac and/or fundic mucosa without intestinal metaplasia cannot be defined as 'Barrett's mucosa' [condition that we call 'columnar-lined oesophagus' (CLO)] and up till now, there has been no agreement in literature about the management of this condition. Aurora-A overexpression leads to centrosome amplification, chromosomal instability and aneuploidy in mammalian cells.

Patients and methods: A prospective study was carried out on 28 consecutive patients who presented columnar mucosa above the gastro-oesophageal junction (GOJ) at endoscopy. As controls, two more biopsies were obtained, one on the normal-appearing squamous oesophagus above the GOJ, as far as possible from the columnar mucosa (controls A), and one taken 1 cm below the GOJ (controls B). The Aurora-A and p53 expression levels were analysed respectively by Quantitative Real Time PCR and immunohistochemistry.

Results: Twelve patients were affected by BO (43%) while the other 16 patients (57%) had a CLO. Nine of 28 (32%) cases were focally positive for p53 immunostaining. All the BO/CLO samples were positive for the Aurora-A transcript with regard to controls. Furthermore, 13 of 28 (46%) cases showed overexpression (above the median for the whole group).

Conclusion: Due to the low number of cases, we are not at present able to state that statistically significant quantitative differences in Aurora-A messenger RNA expression exist between CLO and BO cases with and without dysplasia and p53-positive immunostaining. Further studies on a larger number of cases with a follow-up period are necessary in order to establish the risk of progression and the correct management of these subjects.

Key words: Aurora-A overexpression, Barrett's oesophagus, cell cycle, columnar-lined oesophagus, p53 protein

introduction

In recent years, there has been an estimation in North America and Europe of a 40-fold increase in the risk of developing invasive adenocarcinoma of the oesophagus[1]. The proposed model of histological progression of this kind of tumour is a stepwise progression recognised as a metaplasia-dysplasia-adenocarcinoma sequence (MCS) [2]. Moreover, the development of oesophageal adenocarcinoma (OA) is generally closely associated with the presence of a specialised intestinal-type epithelium such as that found in Barrett's oesophagus (BO) [3, 4]. BO occurs in 10%–12% of patients with chronic gastro-oesophageal reflux. Over a period of time, the presence of this stressful agent results in replacement of the normal squamous epithelium by the more acid-resistant columnar epithelium [5]. For this reason, Barrett's mucosa is defined as the endoscopic presence of a metaplastic mucosa with 'goblet cells' in the oesophagus, regardless of the length of the segment [6]. A particular histological condition is when the distal oesophagus showing cardiac and/or fundic mucosa without intestinal metaplasia (IM) cannot be defined as Barrett's mucosa [7] and up till now, there has been no agreement in literature about the management of this condition. Hereafter, we refer to this condition as columnar-lined oesophagus (CLO).

As in the molecular model proposed for colorectal cancer progression [8], several genetic alterations have been reported in the MCS [9]. In particular, p53 alterations have been reported in 5%–10% of cases with indeterminate dysplasia, in 65% of those with low-grade dysplasia, in 75% of cases with

*Correspondence to: Antonio Russo, MD Section of Medical Oncology, Department of Surgery and Oncology, Università di Palermo, Via del Vespro 127, 90127 Palermo, Italy. Tel: +39-091-6552500; Fax: +39-091-6554529; E-mail: lab-oncobiologia@usa.net

†Both authors have contributed equally to this work.

high-grade dysplasia and in 50%–90% of OA [10–12]. The p53 gene codifies for a nuclear phosphoprotein acting as a transcriptional regulator which prevents proliferation of damaged cells, in some cases inducing them to apoptosis [13]. In most normal tissues, the wild-type p53 protein is constitutively expressed at low levels because of a short half-life [14] due to rapid degradation, but it may accumulate in the cell as a result of several types of stress, such as DNA damage, hypoxia, loss of normal growth and survival signals, acidity and inflammatory processes [15], which may occur in different physiological or pathological situations, including tumorigenesis. In response to these situations of stress, p53 becomes stabilised and accumulates mostly in the nucleus. By regulating the expression of a number of genes, p53 then induces cell cycle arrest and/or apoptosis [16]. In Barrett's mucosa, as a consequence of oxidative DNA damage caused by gastro-oesophageal reflux, there is an increased percentage of cells unable to carry out DNA repair; this is sometimes sequentially followed by p53 gene mutation and protein accumulation, DNA aneuploidy, dysplasia and carcinoma [17].

A new important gene correlated to cancer progression is Aurora-A also known as serine threonine kinase 15 (STK15), BTAK, Aurora kinase A, Aurora-2 or AIKI. This gene is a member of the Aurora/Ip11p family and is located in chromosome 20q13. Aurora-A is an important kinase-encoding gene involved in centrosome duplication and distribution; its overexpression leads to centrosome amplification, chromosomal instability and aneuploidy in mammalian cells [18]. Moreover, Aurora-A appears to be regulated by both phosphorylation and dephosporylation [19]. Aurora-A overexpression has been found in many tumour cells and tissues including breast cancer [20], gastric cancer [21], colorectal cancer [22], bladder cancer [23], pancreatic cancer [24], ovarian cancer [25], prostate cancer [26] and oesophageal squamous-cell carcinoma [27]. Up till now, however, there have not been any studies regarding Aurora-A expression alterations in precancerous forms such as BO or CLO, a condition probably preceding BO.

Finally, recent studies have reported that Aurora-A and p53 activities might be related by the phosphorylation of p53 at serine 215. This interaction would bring about the abrogation of p53 DNA binding and transactivation activities and induce the cells to escape apoptosis and cell cycle arrest [28, 29].

The aim of this study was to assess in a consecutive series of 28 cases showing columnar mucosa in the oesophagus at endoscopy, biomolecular alterations, such us Aurora-A overexpression and p53 expression, which precede the histological phenotype and which may help in the choice of the clinical management of these patients.

materials and methods

patient features

A prospective study was carried out on 28 consecutive patients, *Helicobacter pylori* (Hp) free and with no history of previous Hp infection, who presented columnar mucosa above the gastro-oesophageal junction (GOJ) at endoscopy. The GOJ was identified as the point at which the tubular oesophagus changes to became a sack-like structure. The presence of hiatus hernia was noted. The patients had been referred to the Oesophageal Surgical Unit, Department of Oncology, University of Palermo for an evaluation of their suspected gastro-oesophageal reflux disease (GORD). They all complained of one or more symptoms such as heartburn, regurgitation, dysphagia, otolaryngological symptoms and asthma.

tissue handling

After informed consent, four-quadrant biopsies every 2 cm, beginning at the top of the endoscopic segment of the columnar mucosa above the GOJ, were taken from each patient and processed within 30 min of biopsy. As controls, two more biopsies were obtained, one on the normal-appearing squamous oesophagus above the GOJ, as far as possible from the columnar mucosa (Controls A), and one taken 1 cm below the GOJ (Controls B). All the histological analysis was carried out by one of the authors (CD). All biopsies were bisected, one half of each sample was processed for pathological examination, and the remaining half of the sample pool was immediately frozen and stored at −80°C for subsequent biomolecular analysis. For the pathological examination, the samples were fixed in 10% buffered formalin and embedded in paraffin and 5 μ sections were obtained at five different levels and stained with haematoxylin and eosin and Alcian blue periodic acid–Schiff (PAS) stain, in order to facilitate the identification of goblet cells. IM was defined as the presence of intestinal-type goblet cells stained blue with Alcian blue PAS stain.

p53 immunostaining

Immunohistochemical studies were carried out by means of the avidin–biotin complex technique. The primary monoclonal antibodies used in this study were p53 (clone DO7) obtained from Novocastra laboratories. Sections were cut from 10% formalin-fixed, paraffin-embedded materials and then deparaffinised in xylene and re-hydrated through alcohol. The immunohistochemical assay was carried out according to the manufacturer's instructions of Universal LSAAB (Dako, Glostrup, Denmark). In order to improve the immunostaining, samples were digested with 0.1% trypsin and microwaved in 10 mM citrate buffer (pH 6.0) before incubation. As a positive control for p53, the immunostaining was carried out on sections of rectal adenocarcinoma which had proved to be positive in previous assessments for the above-mentioned antibodies. Finally, negative controls without primary antibodies were included in each run of immunohistochemistry. We considered a nuclear staining pattern as positive for p53 immunostaining. All cases were revised by the two pathologists (CD and AF) and only cases in which there was interobserver agreement about the presence of low-grade dysplasia were considered as dysplastic. Moreover, in keeping with previous reports on p53 values in interobserver agreement of low-grade Barrett's dysplasia diagnosis and in the correlation with disease progression, the presence of at least focal p53 expression was used to confirm the low-grade dysplasia [30–32].

RNA extraction and complementary DNA synthesis

Total RNA was extracted with the use of the Rneasy Minikit (Qiagen, Hilden Germany) according to the manufacturer's instructions. The extracted RNA was stored at −80°C until further use. RNA integrity was verified on the Agilent 2100 bioanalyzer with the use of the RNA 6000 Nano assay protocol (Agilent Technologies, Palo Alto, CA). Only samples with an RNA integrity number between 8 and 10 were analysed. For complementary DNA (cDNA) synthesis 5 μg of total RNA were reverse transcribed in a final volume of 50 μl with a High Capacity cDNA Archive kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The samples were incubated for 10 min at 25°C and for 2 h at 37°C on the GeneAmp 9700 Applied Biosystem (Applied Biosystems).

quantitative determination of Aurora-A by Real Time RT-PCR

Real Time RT-PCR was carried out with the ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems) using the TaqMan method. Quantification was carried out with the use of the threshold cycle (Ct) value. For the detection of Aurora-A and for normalisation the following predesigned primer and probe set were used: assay-on-demand Gene Expression Product, number Hs01590514_m1, AURKA and assay-on-demand Gene Expression Product, number 4333763F, Hu-PPIA, (Applied Biosystems). For the PCR, 100 ng of cDNA in a final volume of 50 μl of TaqMan Universal PCR Master Mix (Applied Biosystems) was used according to the manufacturer's guidelines. Each sample was analysed in triplicate and the mean quantity of each triplicate calculated by the ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems). In our study, the comparative Ct method was used to quantify the relative gene expression with the formula $2^{-\Delta\Delta Ct}$, using cyclophilin A (Hu-PPIA) as the endogenous control. Two different control samples were used as calibrators. Specifically, for each patient the analysis was conducted in triplicate by comparing the Aurora-A expression levels of columnar mucosa above the GOJ, respectively toward normal-appearing squamous oesophagus (control A), with those 1 cm below the GOJ columnar mucosa (control B) and then making a comparison between the two controls.

results

histological and p53 immunohistochemical results

Table 1 shows all the pathological characteristic of the biopsies investigated. Patients were, principally, male (19/28, 68%) with a mean age of 46 (range 27–61). Twelve patients were affected by BO (43%) (Figure 1A) while the others 16 patients (57%) had a CLO. All the CLO samples lacked Alcian blue-positive goblet cells at histology (Figure 1B) and weak Alcian blue positivity was present in a few columnar cells only in some cases (Figure 1C). The control A cases consisted of histologically normal-appearing squamous mucosa. The control B samples consisted of normal-appearing fundic mucosa without goblet cells (Figure 1D). Nine of 28 (32%) cases [five of 12 BO (42%) and four of 16 CLO (25%)] showed some histological alterations such as pale and inconspicuous cytoplasm of the columnar cells; reduction of the cytoplasmic mucous production and enlarged, rounded, vescicular nuclei with evident nucleoli, occurring without inflammation and indicative of the so called 'low-grade dysplasia type II' or 'hyperplastic dysplasia'. They were focally positive for p53 immunostaining (Figure1E, F and G). Control A cases showed mild p53-positive immunostaining only in the basal layer of squamous epithelium. Control B cases were devoid of dysplastic alterations and were p53 negative (Figure 1H)

Aurora-A mRNA expression

RNA extracted from 28 samples of columnar mucosa '1 cm above GOJ' (consisting both of BO and CLO samples) and from the two controls was analysed for Aurora-A messenger RNA expression by Real Time RT-PCR. All the BO/CLO samples were positive for the Aurora-A transcript with regard to controls. In particular, the comparison of the two controls showed the same Aurora-A expression value close to 1 for all samples while all the BO/CLO samples had a higher

Table 1. Clinicopathological features of Barrett's oesophagus patients

Clinicopathological features	Barrett's oesophagus tissue patients, *n* (%)
Age (years)	
≤50	18 (64)
>50	10 (36)
Mean age in years (range)	47 (27–61)
Sex	
Male	19 (68)
Female	9 (32)
Histotype	
Columnar-lined oesophagus	16 (57)
Barrett's oesophagus	12 (43)
Dysplasia	
Present	9 (32)
Absent	19 (68)
Total	28

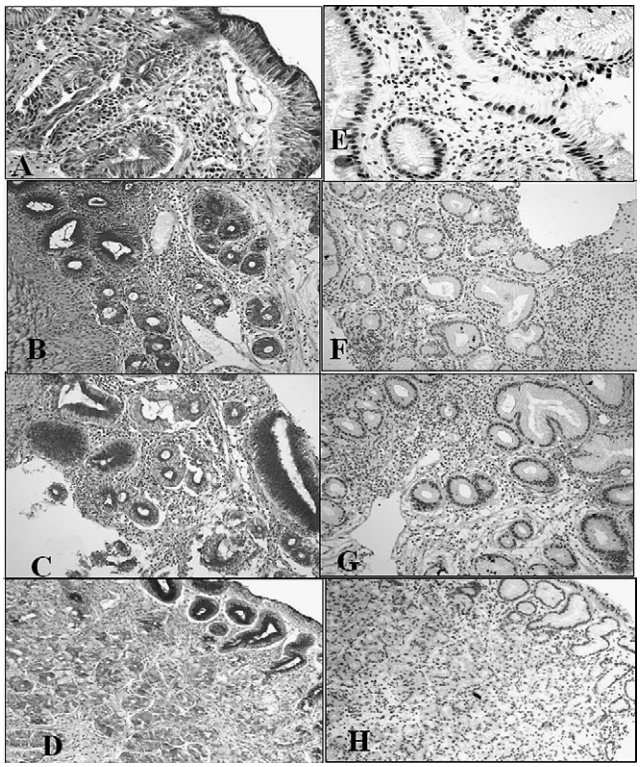

Figure 1. (A) Barrett's case: Alcian blue-positive goblet cells and low-grade dysplasia. (B) Columnar-lined oesophagus (CLO) case: columnar mucosa devoid of Alcian blue-positive goblet cells. (C) CLO case: only weak Alcian blue positivity was present in a few columnar cells, lacking of goblet cell morphology. (D) Control B case: normal-appearing fundic mucosa, without goblet cells and lacking of dysplasia. (E–H) p53 immunostaining is positive both in Barrett's case (E) and in CLO cases (F and G) and is absent in control B case (H). (A–D) Alcian blue periodic acid–Schiff staining; (E–H) p53 immunostaining. Original magnification: (A–E) ×200; (B–D and F–H) ×100.

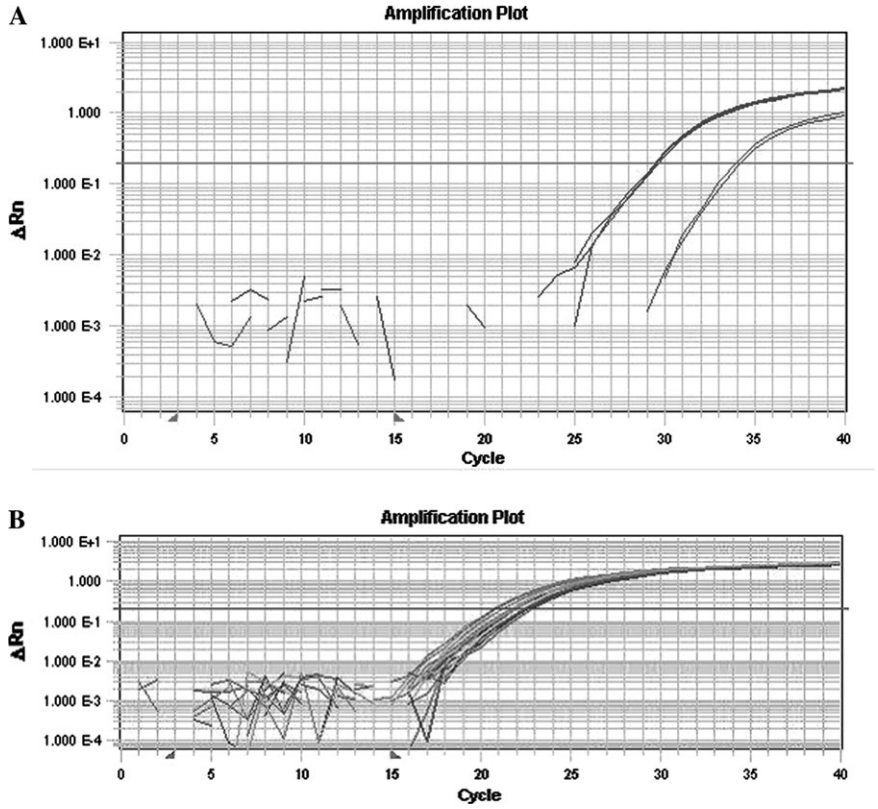

Figure 2. (A) Example of Aurora kinase A expression levels in Barrett's oesophageal (11BO) and control A ($11C_A$) of the case number 11; (B) Cyclophillin A (peptidylprolyl isomerase A [PPIA]) expression levels in all 22 samples analysed.

Aurora-A expression level when compared both to the control A and to the control B cases. Furthermore, 13\28 (46%) cases showed overexpression (above the median for the whole group). Figure 2 shows an example of Real Time RT-PCR experiment of Aurora-A expression respectively in 1 cm above GOJ columnar mucosa case and control A, belonging to the same patient and the peptidylprolyl isomerase A (PPIA) expression levels of all samples included in this study.

discussion

Most oesophageal tumours are adenocarcinomas (OA) which generally arise from a MCS. Although this sequence has been fully studied and characterised, a small percentage of OA arise from BO. This is a precancerous form associated with chronic GORD where squamous epithelium is replaced by metaplastic columnar epithelium. The natural history of the sequence BO-OA is not well known but it is likely that the store of multiple genetic alterations gives rise to Barrett's oesophagus-a non-invasive neoplasia-oesophagus adenocarcinoma (BO-NIN-OA) sequence. The diagnosis of BO is mainly based on the presence of IM and goblet cells [33]. Nowadays, although it is well known that BO-carrier patients show 30–125 times more risk for developing OA than the normal population, it is still difficult to discriminate the BO patients with a major risk of developing OA. The application of a rigid follow-up of BO patients after the first endoscopic–histological diagnosis is the only way to identify the presence of OA at an early stage. In certain patients, moreover, for instance in those with CLO, the absence of intestinal goblet cells does not permit the inclusion of such patients in follow-up programmes, which gives rise to an unclear clinical management of these cases. For all these reasons, the aim of this study is to identify the presence of Aurora-A and p53 alterations in specific forms such us CLO and BO with the object of assessing in these forms the biomolecular alterations that precede the histological phenotype and that may help in the choice of the clinical management of such cases. These analyses have been conducted not by means of the identification of the genetic alteration but by the study of the different Aurora-A mRNA and p53 protein expression levels, in order to determine their effect directly on the RNA and/or protein.

Several sporadic tumours often show chromosomal aberrations that precede genetic alterations and that might give rise to histological changes and then to cancer progression. The presence of these chromosomal alterations has, in fact, been related to the invasive tumour. The chromosomal aberration occurs principally in dividing cells during the mitotic event when the chromosomes are aligned in a metaphasic plate and are about to be segregated into two daughter cells. Each step of this multiphase process is strictly regulated by several checkpoints and by different proteins with a specific role in chromosome condensation, alignment and segregation. The alterations in mitosis will give rise to abnormal chromosome segregation and then to cells with

aberrant genetic panels. This study focused on the analysis of two proteins playing two important key roles in the cell cycle: Aurora-A, also known as 'a guardian of the pole', and p53, previously known as 'guardian of the genome'. The first is a serine threonine kinase which, when activated by phosphorylation, is involved in several phases of mitosis such us centrosome maturation, centrosome separation, mitotic entry, bipolar spindle assembly and chromosome alignment on the metaphase plate [34]. The Aurora-A gene is located at chromosome 20q13, which is commonly amplified in various epithelial malignant tumours, including breast, colon, bladder, ovarian and pancreatic cancer and the levels of Aurora-A mRNA and protein are also increased in such tumours [34]. Contrasting data are reported on the Aurora-A role in the histological grade of human tumours [35–37]. Some studies, in fact, have indicated that Aurora-A overexpression is significantly associated with higher grade tumours and poor prognosis, while others report that the activation and overexpression of Aurora-A is more frequently detected in early-stage human ovarian cancers. Up-regulation of Aurora-A mRNA and protein were analysed by semiquantitative reverse transcription PCR and immunohistochemistry in patients affected by human oesophageal squamous cell carcinoma (OSCC) and in paired normal tissues [27, 38]. These studies have reported that Aurora-A expression, in terms of both mRNA and protein, is elevated in OSCC tissues and that it is correlated with distant lymph node metastasis and tumour invasion. Furthermore, Tanaka et al. [20] report that the up-regulation of Aurora-A protein is an independent prognostic factor. Our study is the first study in which the different Aurora-A expression level is analysed in the precancerous form of the oesophagus such as in BO and in CLO, a condition probably preceding BO.

The presence of IM with goblet cells is considered the most significant change for the diagnosis of BO, a fact emphasised by the dictum 'no goblets, no Barrett's' [33]. The meaning of CLO devoid of goblet cells or showing only a few Alcian blue positive, columnar cells in the malignant transformation of BO is still debated [39–42] and there will probably be no follow-up of these patients. Sometimes, CLO has been considered a function of sampling error [43] that might be reduced by increasing the number of biopsies at the initial diagnostic endoscopy [44], by the repetition of sampling and/or by histological assessment of more numerous sections. In our study, all of the 28 '1 cm above the GOJ' samples, both consisting of BO and of CLO cases, had a major Aurora-A expression compared with the paired normal squamous oesophagus and normal mucosa 1 cm below the GOJ used as controls, while the comparison between paired control A and control B cases always shows the same low expression levels (data not shown). These differences in Aurora-A expression levels in columnar mucosa above and below GOJ make it unlikely, at least for most of the CLO cases, that a sampling mistake is involved and indicate that CLO may not be a completely benign mucosa, and 'in addition to the goblet cells, the non-goblet elements may also be involved in the malignant transformation of BO' [39]. These preliminary results indicate that a surveillance program, at least in this phase of the study, should be indicated for such patients. Unfortunately, no data of follow-up are available to assess the role of Aurora-A overexpression in BO-OA progression. It is noteworthy that in our study, Aurora-A mRNA was over-expressed in all the cases, whether or not intestinal goblet cells, dysplasia and p53 positivity were present. In this regard, it is important to consider that genetic alterations are events preceding phenotype manifestation and are not always immediately reflected in histological alterations. Due to the low number of cases, we are not at present able to state that statistically significant quantitative differences in Aurora-A mRNA expression exist between CLO and BO cases with and without dysplasia and p53-positive immunostaining. This matter will be the object of our further studies on a larger number of cases with a follow-up period in order to establish the risk of progression and the correct management of these subjects.

references

1. Spechler SJ, Robbins AH, Rubins HB et al. Adenocarcinoma and Barrett's esophagus. An overrated risk? Gastroenterology 1984; 87(4): 927–933.
2. Jankowski JA, Wright NA, Meltzer SJ et al. Molecular evolution of the metaplasia-dysplasia-adenocarcinoma sequence in the esophagus. Am J Pathol 1999; 154(4): 965–973.
3. Barrett NR. Chronic peptic ulcer of the oesophagus and 'oesophagitis'. Br J Surg 1950; 38(150): 175–182.
4. Lortat-Jacob JL. L'endobrachy-oesophage. Ann Chir 1957; 11: 1247–1254.
5. Levine DS, Rubin CE, Reid BJ, Haggitt RC. Specialized metaplastic columnar epithelium in Barrett's esophagus. A comparative transmission electron microscopic study. Lab Invest 1989; 60(3): 418–432.
6. Sampliner RE. Updated guidelines for the diagnosis, surveillance, and therapy of Barrett's esophagus. Am J Gastroenterol 2002; 97: 1888–1895.
7. Takubo K, Vieth M, Aryal G et al. Islands of squamous epithelium and their surrounding mucosa in columnar-lined esophagus: a pathognomonic feature of Barrett's esophagus? Hum Pathol 2005; 36(3): 269–274.
8. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990; 61(5): 759–767.
9. McManus DT, Olaru A, Meltzer SJ. Biomarkers of esophageal adenocarcinoma and Barrett's esophagus. Cancer Res 2004; 64(5): 1561–1569.
10. Casson AG, Mukhopadhyay T, Cleary KR et al. p53 gene mutations in Barrett's epithelium and esophageal cancer. Cancer Res 1991; 51: 4495–4499.
11. Younes M, Lebovitz RM, Lechago LV, Lechago J. p53 protein accumulation in Barrett's metaplasia, dysplasia and carcinoma: a follow-up study. Gastroenterology 1993; 105: 1637–1642.
12. Hamelin R, Flejou JF, Muzeau F et al. TP53 mutations, and p53 protein immunoreactivity in malignant and premalignant Barrett's oesophagus. Gastroenterology 1994; 107: 1012–1018.
13. Ryan KM, Phillips AC, Vousden KH. Regulation and function of the p53 tumor suppressor protein. Curr Opin Cell Biol 2001; 13(3): 332–337.
14. Brown JP, Pagano M. Mechanism of p53 degradation. Biochim Biophys Acta 1997; 1332(2): 1–6.
15. Takashi T, Nakamura Y. The role of p53-target genes in human cancer. Crit Rev Oncol Hematol 2000; 33: 1–6.
16. Ryan KM, Phillips AC, Vousden KH. Regulation and function of the p53 tumor suppressor protein. Curr Opin Cell Biol 2001; 13(3): 332–337.
17. Younes M, Lechago J, Chakraborty S et al. Relationship between dysplasia, p53 protein accumulation, DNA ploidy, and Glut1 overexpression in Barrett metaplasia. Scand J Gastroenterol 2000; 35(2): 131–137.
18. Dutertre S, Descamps S, Prigent C. On the role of aurora-A in centrosome function. Oncogene 2002; 21(40): 6175–6183.
19. Meraldi P, Honda R, Nigg EA. Aurora kinases link chromosome segregation and cell division to cancer susceptibility. Curr Opin Genet Dev 2004; 14: 29–36.

20. Tanaka T, Kimura M, Matsunaga K et al. Centrosomal kinase AIK1 is overexpressed in invasive ductal carcinoma of the breast. Cancer Res 1999; 59: 2041–2044.
21. Sakakura C, Hagiwara A, Yasuoka R et al. Tumour-amplified kinase BTAK is amplified and overexpressed in gastric cancers with possible involvement in aneuploid formation. Br J Cancer 2001; 84: 824–831.
22. Bischoff JR, Anderson L, Zhu Y et al. A homologue of Drosophila Aurora kinase is oncogenic and amplified in human colorectal cancers. EMBO J 1998; 17: 3052–3065.
23. Sen S, Zhou H, Zhang RD et al. Amplification/overexpression of a mitotic kinase gene in human bladder cancer. J Natl Cancer Inst 2002; 94: 1320–1329.
24. Zhu J, Abbruzzese JL, Izzo J et al. AURKA amplification, chromosome instability, and centrosome abnormality in human pancreatic carcinoma cells. Cancer Genet Cytogenet 2005; 159: 10–17.
25. Gritsko TM, Coppola D, Paciga JE et al. Activation and overexpression of centrosome kinase BTAK/Aurora A, in human ovarian cancer. Clin Cancer Res 2003; 9: 1420–1426.
26. Pihan GA, Purohit A, Wallace J et al. Centrosome defects can account for cellular and genetic changes that characterize prostate cancer progression. Cancer Res 2001; 61: 2212–2219.
27. Tanaka E, Hashimoto Y, Ito T et al. The clinical significance of Aurora-A/STK15/BTAK expression in human esophageal squamous cell carcinoma. Clin Cancer Res 2005; 11(5): 1827–1834.
28. Haruki N, Harano T, Masuda A et al. Persistent increase in chromosome instability in lung cancer: possible indirect involvement of p53 inactivation. Am J Pathol 2001; 159: 1345–1352.
29. Liu Q, Kaneko S, Yang L et al. Aurora-A abrogation of p53 DNA binding and transactivation activity by phosphorylation of serine 215. J Biol Chem 2004; 279: 52175–52182.
30. Bian YS, Osterheld MC, Bosman FT et al. p53 gene mutation and protein accumulation during neoplastic progression in Barrett's esophagus. Mod Pathol 2001; 14: 397–403.
31. Skacel M, Petras RE, Rybicki LA et al. p53 expression in low grade dysplasia in Barrett's esophagus: correlation with interobserver agreement and disease progression. Am J Gastroenterol 2002; 97(10): 2508–2513.
32. Weston AP, Banerjee SK, Sharma P et al. p53 protein overexpression in low-grade dysplasia (LGD) in Barrett's esophagus. Immunohistochemical marker predictive of progression. Am J Gastroenterol 2001; 96: 1355–1362.
33. Batts KP. Barrett esophagus–more steps forward. Hum Pathol 2001; 32: 357–359.
34. Marumoto T, Zhang D, Saya H. Aurora-A—a guardian of poles. Nat Rev Cancer 2005; 5: 42–50.
35. Gritsko TM et al. Activation and overexpression of centrosome kinase BTAK/Aurora-A in human ovarian cancer. Clin Cancer Res 2003; 9: 1420–1426.
36. Jeng YM, Peng SY, Lin CY, Hsu HC. Overexpression and amplification of Aurora-A in hepatocellular carcinoma. Clin Cancer Res 2004; 10: 2065–2071.
37. Miyoshi Y, Iwao K, Egawa C, Noguchi S. Association of centrosomal kinase STK15/BTAK mRNA expression with chromosomal instability in human breast cancers. Int J Cancer 2001; 92: 370–373.
38. Tong T, Zhong Y, Kong J et al. Overexpression of Aurora-A contributes to malignant development of human esophageal squamous cell carcinoma. Clin Cancer Res 2004; 10(21): 7304–7310.
39. Chaves P, Cardoso P, de Almeida JC et al. Non-goblet cell population of Barrett's esophagus: an immunohistochemical demonstration of intestinal differentiation. Hum Pathol 1999; 30(11): 1291–1295.
40. Chandrasoma P. Controversies of the cardiac mucosa and Barrett's oesophagus. Histopathology 2005; 46(4): 361–373.
41. Lombard C. Controversies of the cardiac mucosa and Barrett's oesophagus. Histopathology 2006; 49(1): 97–98.
42. Chandrasoma P. Controversies of the cardiac mucosa and Barrett's oesophagus. Histopathology 2006; 49(1): 97–98.
43. Goldblum JR, Lee RG. Esophagus. In Stenberg (ed): Diagnostic Surgical Pathology. Philadelphia, PA: Lippincott Williams & Wilkins 2004.
44. Coad RA, Shepherd NA. Barrett's oesophagus: definition, diagnosis and pathogenesis. Curr Diagn Pathol 2003; 9(4): 218–227.