Early Changes in Carcinogenesis of Colorectal Adenomas

S Toru[1], B Bilezikçi[2]

ABSTRACT

Objectives: *Adenocarcinoma of the colon and rectum is the third most common cause of cancer deaths and the sixth most common cancer in the world. Adenomas are benign neoplastic lesions which can be transformed into carcinomas, but this is usually not the case. There should be some risk factors which lead to the development of carcinomas into adenomas. The aim of this study is to find out the early changes and high risk factors related to carcinogenesis in colonic polyps.*

Methods: *In this study, we reviewed nearly 1000 colonoscopic biopsies and chose 72 biopsies. We developed three groups (tubular adenomas group 1, villous adenomas group 2, normal mucosa group 3); each group had 24 different biopsies. P53, Ki-67, bcl-2, cyclin D1, E-cadherin, c-erb B2 immunohistochemistry and human papillomavirus (HPV)* in-situ *hybridization were used for analysis.*

Results: *Five of the seventy-two cases were positive in HPV* in-situ *analysis. Four of them were villous adenomas and one was a tubular adenoma. Ki-67 expression was limited only to crypts in group 3 but in groups 1 and 2, Ki-67 expression was seen both in crypt epithelium and surface epithelium. Cyclin D1, c-erb B2, bcl-2 expression was significantly increased in neoplastic polyps.*

Conclusion: *Ki-67 expression, both in the crypt and surface epithelium, and cyclin D1, c-erb B2, bcl-2 over-expression may be a clue of dysplastic epithelium and if the role of HPV is elucidated and shown to be important in colonic carcinogenesis, then vaccination might prevent carcinogenesis caused by HPV.*

Keywords: Adenomatous polyps, carcinogenesis, HPV

Cambios Tempranos en la Carcinogénesis de los Adenomas Colorectales

S Toru[1], B Bilezikçi[2]

RESUMEN

Objetivos: *El adenocarcinoma del colon y recto es la tercera causa más común de muertes por cáncer y el sexto tipo de cáncer más común en el mundo. Los adenomas son lesiones neoplásicas benignas que pueden transformarse en carcinomas, pero éste normalmente no es el caso. Debe haber algunos factores de riesgo que conducen al desarrollo de carcinomas en adenomas. El objetivo de este estudio es averiguar los cambios tempranos y los factores de alto riesgo relacionados con la carcinogénesis en los pólipos colónicos.*

Métodos: *En este estudio, revisamos casi 1000 biopsias colonoscópicas y escogimos 72 biopsias. Desarrollamos tres grupos (grupo 1: adenomas tubulares, grupo 2: adenomas vilosos, grupo 3: mucosa normal); cada grupo tuvo 24 biopsias diferentes. Para el anílisis se utilizaron la inmunohistoquímica de P53, Ki-67, bcl-2, ciclina D1, E-cadherina, y c-erb B2, así como la hibridación in situ para la detección del virus del papiloma humano (VPH)*

Resultados: *Cinco de setenta y dos casos resultaron positivos en el análisis del VPH in-situ. Cuatro de ellos fueron adenomas vilosos, de los cuales uno era un adenoma tubular. La expresión Ki-67 está limitada sólo a las criptas en el grupo 3, pero en los grupos 1 y 2, la expresión Ki-67 se observó tanto en el epitelio de la cripta como en el epitelio de la superficie. La expresión de la ciclina D1, c-erb B2, y bcl-2 se halla significativamente aumentada en los pólipos neoplásicos.*

Conclusión: *La expresión de Ki-67 tanto en el epitelio de la cripta como de la superficie, y la sobre-expesión de la ciclina D1, c-erb B2, bcl-2 puede ser una clave para el epitelio displásico, y si se aclara y demuestra que el papel del VPH es importante en la carcinogénesis colónica, entonces la vacunación podría prevenir los carcinogénesis inducidos por el VPH.*

From: [1]Akdeniz University Pathology Department, Antalya, Turkey and [2]Baskent University Pathology Department, Ankara, Turkey

Correspondence: Dr S Toru, Akdeniz University Pathology Department, Antalya, Turkey. E-mail: serap_toru@yahoo.com

Palabras claves: Pólipos adenomatosos, carcinogénesis, VPH

West Indian Med J 2012; 61 (1): 11

INTRODUCTION

Adenocarcinoma of the colon and rectum is the third most common cause of cancer deaths in the world (1) and it is the sixth most common cancer (2). Moreover, it persists as a significant cause of cancer mortality in Turkey. Colorectal carcinogenesis develops as a result of genetic changes in cell growth, differentiation and apoptosis (3). Although the pathogenesis of this disease is a multistep process, it is becoming better understood at the molecular level, but there is still controversy about its aetiology (1). Genetic (like familial polyposis) and dietary factors are well-known aetiologic agents of colonic carcinogenesis. The dietary factors most closely associated with increased colorectal cancer rates are low intake of unabsorbable vegetable fibre and high intake of refined carbohydrates and fat. Although these associations are clear, the mechanistic relationship between diet and risk remains poorly understood. However, it is theorized that reduced fibre content leads to decreased stool bulk and altered composition of the intestinal microbiota. This change may increase synthesis of potentially toxic oxidative by-products of bacterial metabolism (4). Nowadays, the most talked about probable factors are oncogenic viral agents like human papillomavirus [HPV] (5). Over 90% of adenocarcinomas are generated through adenomas which are benign neoplastic lesions (4). Adenomas can be classified as tubular, tubulovillous, or villous based on their architecture. These categories, however, have little clinical significance in isolation. Tubular adenomas tend to be small, pedunculated polyps composed of small, rounded, or tubular glands. In contrast, villous adenomas, which are often larger and sessile, are covered by slender villi. Tubulovillous adenomas have a mixture of tubular and villous elements. Although villous adenomas contain foci of invasion more frequently than tubular adenomas, villous architecture alone does not increase cancer risk when polyp size is considered (6). So there could be oncogenic and environmental factors with alteration of cell proliferation and apoptosis that might lead adenomas to become carcinomas.

Briefly summarized, the main factors influencing development and progress of carcinomas are the following: cyclin D1, bcl-2 (p53 dependent and non-dependent), Ki-67, c-erb B2 and p53 which are the pathways affected in colorectal carcinogenesis (7−10). E-cadherin over-expression is said to increase the risk of metastasis from colonic carcinomas, bcl-2 over-expression may be the predictive factor of colonic carcinogenesis (1). There are many articles which indicate that high risk HPV types are found more in high grade colorectal carcinomas than low grade ones (11−18).

Ki-67 proliferation index is related to cell proliferation; Hamilton and Aaltonen stated that Ki-67 over-expression is seen in adenomas with high grade dysplasia (2). Andersen *et al* mentioned that the Ki-67 proliferation index is higher in dysplastic mucosa than normal mucosa (9). Likewise, we discovered that Ki-67 expression is seen both in crypt epithelium and surface epithelium in adenomas. Moreover, among the colorectal carcinomas, c-erb B2 and p53 expression are determined mostly in rectal carcinomas. According to Tavangar *et al,* over fifty per cent of carcinomas with over-expression of c-erb B2 and p53 have vascular invasion and lymph node metastasis (10). However, in the present study, we looked at adenomas instead of carcinomas, and assessed that c-erb B2 expression increases the risk of neoplastic polyp genesis 5.1 times. Concerning environmental factors, HPV type 16 is seen more in the colorectal carcinomas than in the normal mucosa around carcinomas (13). Zhu *et al* found that HPV was more likely to be positive in colorectal carcinomas than surrounding normal mucosa and it was negative in the control group with normal mucosa (17). We found that HPV was not only negative in normal mucosa but that it was also positive in five of all adenomas.

The aim of this study is to compare the effect of HPV, p53, c-erb B2, E-cadherin, bcl-2, cyclin D1 expression and Ki-67 proliferation index in tubular and villous adenomas with the normal colonic mucosa and to show the distribution of these values according to type of polyps and the age of the patient and to find out their probable role in carcinogenesis.

MATERIAL AND METHODS

We reviewed nearly 1000 colonoscopic biopsies between 1995 and 2007 from the archive of Baskent University Ankara Hospital Pathology department. Archival, formaldehyde-fixed, paraffin embedded tissue blocks of tubular adenoma, villous adenoma and normal colon mucosa biopsies were chosen for this study. Serrated polyps were excluded because the carcinogenesis pathway is different from conventional adenomatous polyps.

We developed three groups (tubular adenomas − group 1, villous adenomas − group 2, normal mucosa − group 3), with each group having 24 different biopsies. We took the biopsies which were without *in-situ* nor invasive carcinoma and excluded the biopsies with distinctive inflammation. Part of the colon between the caecum and splenic flexure is the right colon and from splenic flexure to end is the left colon (20). Slices from paraffin blocks were sectioned 3μ thick, examined immunohistochemically using a standard immunoperoxidase method using respective antibodies p53 (DO-7; DAKO Cytomation), Ki-67 (SP6- Neomarkers), bcl-2 (N1587, DAKO), cyclin D1 (SP4-Neomarkers), E-cadherin (MS-1475-R7, Neomarkers), c-erb B2 (MS-730-P1, Neomarkers) and *in-situ* hybridization with HPV antibody (HPV probe-wide spectrum; DAKO Cytomation).

On light microscopy, the markers were evaluated as below:

1- Nuclear Ki-67 expression of 1000 epithelial cells, and proliferation index obtained (21).

2- Nuclear p53 expression; if the expression was less than 10% of epithelial cells – negative; 10−50%, 1+; and over 50%, 2+ (22).

3- Cytoplasmic bcl-2 expression; no expression was accepted as negative, 1−25%, 1+; 26−50%, 2+; and over 50%, 3+ (23).

4- Nuclear cyclin D1 expression in epithelial cells; if the expression is under 5% – negative; 6−25% 1+; 26−50%, 2+; 51%−75%, 3+; and over 76%, 4+ (7).

5- Membranous E-cadherin expression in epithelial cells; under 10% – negative, 10−30%, 1+; over 30%, 2+ (24, 25).

6- Membranous c-erb B2 expression in epithelial cells; no membranous expression or less than 10% of epithelial cells with membranous staining – negative; over 10% but incomplete membranous staining, 1+; over 10% but weak – medium complete membranous staining, 2+, over 10% but strong membranous staining, 3+ (26).

7. If nuclear HPV staining was seen in at least one cell, it was assumed as positive.

Statistically, SPSS software (Statistical Package for the Social Sciences, version 11.0, SSPS INC, Chicago, IL, USA) was used. Group results were examined with variant analysis, Tamhane's T2 test, Ki-Square test, Kendall's rank correlation and logistic regression analysis.

RESULTS

Average age of all cases are 62.3 (± 15.7), group 1, 62.7 (± 10.8); group 2, 68.3 (± 12.7) and group 3 55.9 (± 20.2). The distribution of average age between the groups is statistically significant ($p < 0.05$). This is because group 3 with normal mucosa has a lower average age than the others (Post Hoc analysis).

In HPV *in-situ* analysis, five of seventy-two cases were positive. It was not statistically significant but all positive cases have neoplastic polyps, four of them were villous adenomas, and one was a tubular adenoma (Fig. 1).

Immunohistochemical examination of Ki-67, E-cadherin and p53 showed no significant differences in all cases ($p > 0.05$). On the other hand, Ki-67 expression was limited only to crypts in group 3 (Fig. 2a) but in groups 1 and 2, Ki-67 expression was seen both in crypt epithelium and surface epithelium (Fig. 2b). This staining pattern shows the dysplastic epithelium.

In groups 2 and 3, cyclin D1 expression is homogeneous but the distribution is heterogeneous in group 1. Cyclin D1 expression (Fig. 3) is significantly increased in neoplastic polyps when compared with normal mucosa ($p < 0.05$). C-erb B2 expression is increased in groups 1 and 2 compared to group 3 (Fig. 4); the difference is statistically significant. In logistic regression analysis, it is shown that C-erb B2 expression is associated with neoplastic polyp genesis, increases the risk of neoplastic polyp genesis 5.1 times and the difference is statistically significant [$p < 0.05$] (Table 1). In Kendall's rank correlation test, c-erb B2 expression has a positive correlation ($r = 0.289$, $p < 0.05$) with neoplastic polyp genesis.

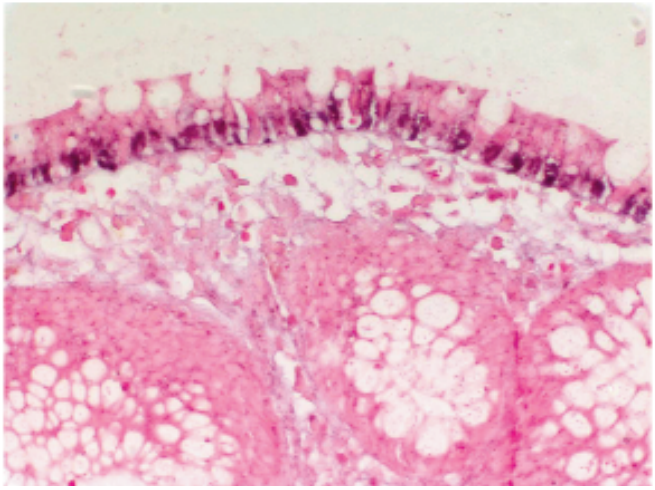

Fig. 1: Nuclear human papillomavirus positivity with *in-situ* hybridization analysis in villous adenoma (x 40).

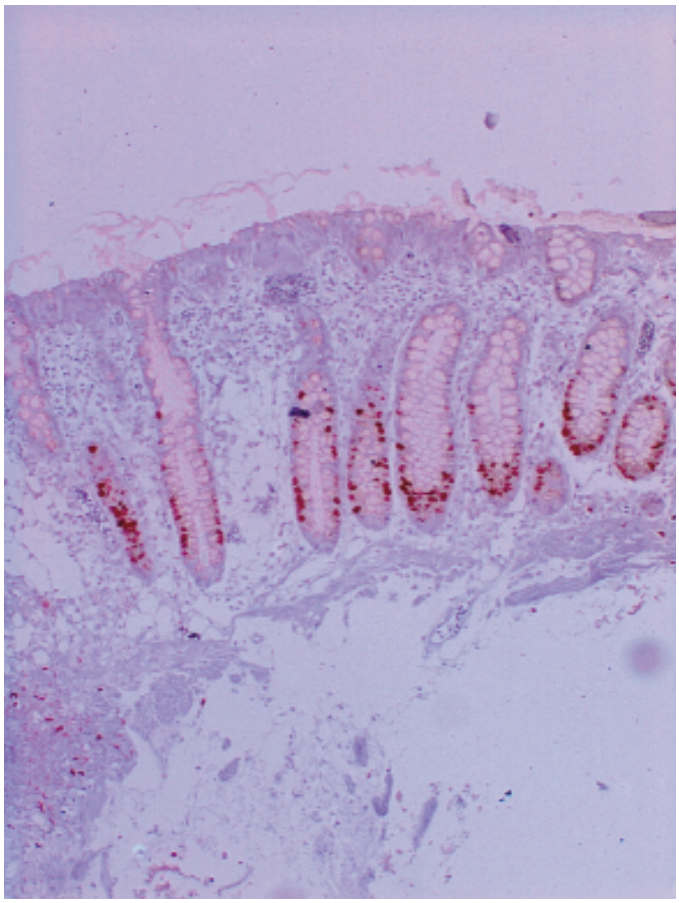

Fig. 2a: Ki-67 expression limited to crypt epithelium in normal mucosa (x 20).

As far as e-cadherin and p53 expression are concerned, there is no statistically significant difference between the

Table 1: Analysis of neoplastic polyp genesis with logistic regression analysis

Step 1				
Constant: -1.374	**beta**	**OR***	**95% CI****	***p***
HPV	8.727	6165.9	0.0−3.77	0.840
Negative/positive Sex	0.138	1.1	0.32−4.04	0.262
Female/male c-erb B2	1.075	2.9	046−18.68	0.255
P53	5.672	290.6	0.0−4.72	0.881
Cyclin D1	0.504	1.6	0.81−3.37	0.166
E-cadherin	0.178	1.2	0.56−2.52	0.640
Ki-67	-0.007	0.9	0.96−1.02	0.628
Bcl-2	0.345	1.4	0.52−3.77	0.492
Age	0.021	1.0	0.98−1.06	0.321
Step 2				
Constant: -0.091	**beta**	**OR***	**95% CI****	***p***
HPV	7.832	2520.1	0.0−3.56	0.763
Negative/positive c-erb B2	1.628	5.1	1.04−24.84	0.04
Cyclin D1	**0.530**	**1.7**	**0.90–3.17**	**0.09**

*OR: Odds Ratio
**CI: Confidence Interval
HPV: Human papillomavirus

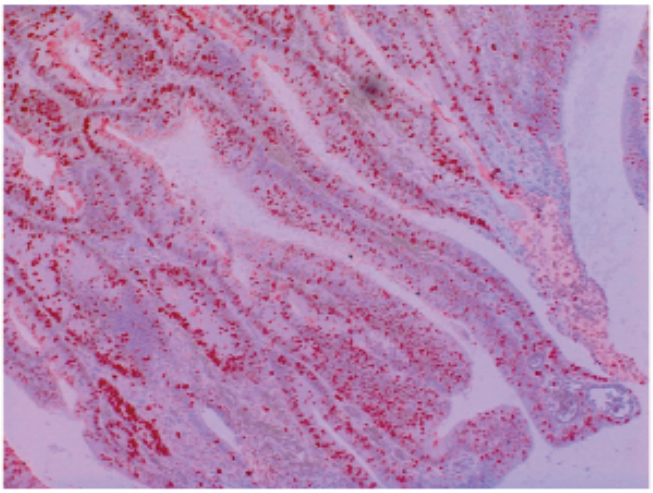

Fig. 2b: Ki-67 expression reaches surface epithelium in villous adenoma (x 10).

groups. Bcl-2 expression is increased in tubular and villous adenomas (Fig. 5) and the difference is statistically significant ($p < 0.05$).

The localization did not have significant difference ($p > 0.05$) according to diagnosis but 75% of tubular adenomas and 79.2% of villous adenomas were in the left colon.

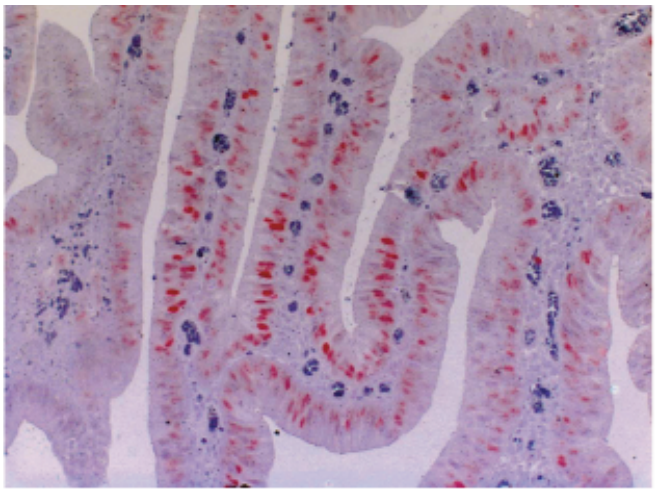

Fig. 3: Nuclear cyclin D1 expression in tubular adenoma (x 20).

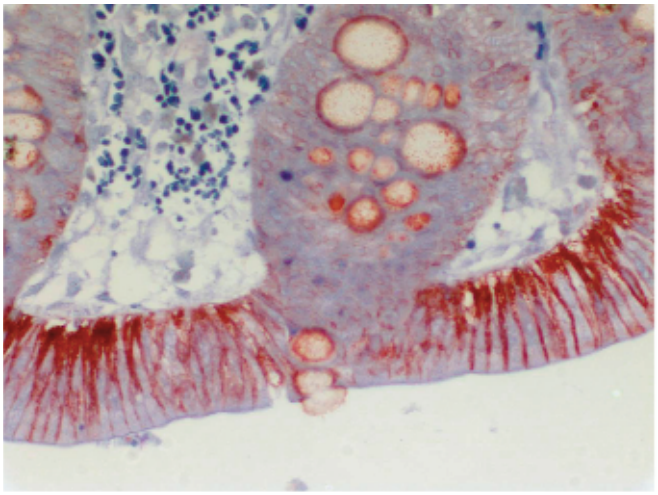

Fig. 4: Granular and membranous c-erb B2 expression in villous adenoma (x 40).

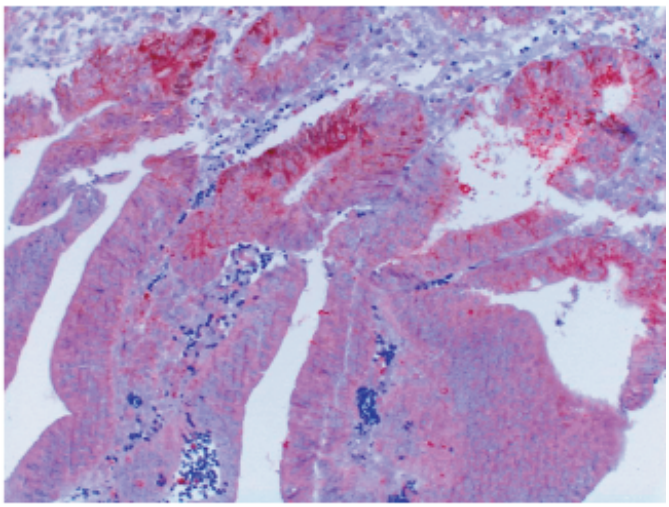

Fig. 5: Cytoplasmic bcl-2 expression in villous adenoma (x 40).

DISCUSSION

Colorectal carcinomas are the third most common carcinomas and the cause of 10% of cancer deaths (28). Colorectal cancer is the sixth most common cancer and persists as a significant cause of cancer mortality in Turkey (29). Colorectal carcinogenesis is generally a slow process spanning decades from cancer initiation to diagnosis. The carcinomas are characterized by the progressive accumulation of genetic abnormalities, including inactivation of tumour suppressor genes and activation of proto-oncogenes. The carcinogenic process requires alterations in the balance between cell renewal and cell death that regulate normal cellular homeostasis in colonic mucosa (30). The close medical follow-up of the tubular and villous adenomas and the early carcinogenetic changes seen in adenomatous polyps could provide early diagnosis and treatment of colonic carcinomas.

In previous studies, it is shown that the probability of colorectal carcinoma increases in old age. In the present study, the average age was significantly higher in patients with adenomas than patients with normal mucosa. If a regular clinical follow-up is made in elderly patients, adenomatous polyps could be treated before they become carcinomatous. Therefore, there could be a cure which provides a higher quality of life for the patients and avoids the high cost of chemotherapy.

It is known that cyclin D1 promotes cell proliferation through the PI3K pathway in colorectal carcinogenesis (31−33). In the present study, cyclin D1 expression was increased in neoplastic polyps when compared with normal mucosa. It is an important regulator of cell cycle progression and can function as a transcriptional co-regulator. The over-expression of cyclin D1 has been linked to the development and progression to cancer (27). Therefore over-expression of cyclin D1 may be a clue for high risk carcinoma transformation in neoplastic polyps. So, cyclin D1 expression and also scoring according to percentage of nuclear expression in surface epithelium, as in the study by Kouraklis *et al* (7), may be a guide for dysplasia and early change in adenomatous polyp.

In the literature, it is said that bcl-2 expression is seen frequently in adenomas, but in 5−55% of carcinomas. There is no relationship between the stage and differentiation of the tumour. So it is believed that bcl-2 expression could have a role in the early stages of carcinogenesis (24, 34). Apoptosis is a basic mechanism in control of over-proliferation of colonic epithelium, and also has a role in eliminating the broken DNA (3). In the present study, bcl-2 expression was statistically significantly higher in neoplastic polyps than normal mucosa. Because of this, the bcl-2 pathway could be one of the early steps of carcinogenesis in neoplastic polyps.

On the other hand, C-erb B2 over-expression is directly associated with colorectal carcinoma (10). In our study, c-erb B2 expression is significantly increased in neoplastic polyps. C-erb B2 is from the family of epidermal growth factors and affects the growing and spreading of the tumour directly. In many studies, it is said that c-erb B2 expressing colorectal carcinomas are mostly advanced stage and recurrence is more commonly seen and in some studies, it is said that chemotherapy may be considered (29, 35). Thus, c-erb B2 over-expression has a prognostic importance in colorectal carcinoma. In the present study, there was an increase in c-erb B2 expression by 5.1 times the development of the neoplastic polyps, thereby increasing the probability of carcinoma.

In the present study, Ki-67 proliferation index increase in neoplastic polyps was not statistically significant but Ki-67 expression of the distribution showed a difference between normal mucosa and neoplastic polyps. In our study, normal mucosa Ki-67 expression is limited in the crypt base, but reaches to the surface in neoplastic polyps. Ki-67 is a proliferation marker so this finding shows that both the crypt epithelium and the surface epithelium are proliferative parts of the colonic epithelium in neoplastic polyps. This staining pattern is pointing out the dysplastic epithelium. Hence, seeing Ki-67 expression both in the crypt base and on the surface might suggest carcinogenesis (9).

According to Furuta *et al,* e-cadherin expression is decreased in colorectal carcinoma (36). In our study, no statistically significant difference was seen in e-cadherin expression between normal mucosa, tubular adenoma and villous adenoma. This finding might indicate that loss of e-cadherin expression is seen in late stages of carcinogenesis because the loss of e-cadherin expression is said to be associated with lymph node metastasis (37) and poor overall survival (38). E-cadherin may play a role in prognosis after carcinogenesis.

In some studies, anal (both squamous and adenocarcinoma) and rectal adenocarcinoma p53 expression is taken into account but no relation with HPV infection exists (4). But in many studies, colorectal carcinomas have an association with HPV infection, especially type 16. In HPV-related carcinogenesis, p53 mutation pathway is not used; reactive $P^{16INK4A}$ over-expression and its initial result CDK4 over-expression and Rb-tumour supressor gene inactivation is seen (13−18). Many studies have been done about the relationship between colorectal carcinoma and HPV infection but few have been done about the relationship between HPV infection and colorectal adenomas. In 1995, Cheng *et al* showed that HPV type 16 infection has a relationship with histological type of colonic adenoma and is significantly increased in villous adenoma; and also the degree of epithelial dysplasia increases with HPV DNA load (39). In the present study, the number of cases was not enough but five cases were HPV positive. All cases were neoplastic polyps; only one case was a tubular adenoma, the other cases were villous adenomas. This finding may point out the role of HPV in colonic adenomatous changes and the higher tendency of transformation to carcinoma. We expect that if the study population number is enlarged, the relationship between HPV infection and villous adenomas could be found. In cervical carcinoma, HPV vaccination is suggested for prophylaxis (40, 41). In recent times, several studies have examined the possible involvement of HPV in non-genital cancers and have investigated the presence of HPV in oe-

sophageal, laryngeal, tonsillar, lung, urothelial, breast and colorectal cancers. However, the role of HPV has been proven only in the pathogenesis of cervical cancers and HPV vaccination is important in prevention (42). In several studies, HPV DNA has been detected more frequently in colorectal malignant specimens compared to matched normal tissues or non-malignant control samples (17, 18, 42−45). The correlation between high-risk HPV infections and anal and cervical carcinoma is widely accepted, but the correlation between HPV and malignancies of other organs such as upper airways, gastrointestinal tract (including colon and rectum) and breast is not well defined (5). The possible involvement of HPV in non-genital cancers and precursor lesions may necessitate the introduction of the vaccine in both boys and girls and vaccination may prevent the carcinogenesis potentially associated with HPV infections.

Finally, in colonic carcinogenesis many precursor lesions and histopathological and immunohistochemical findings like adenomas are found, so it becomes possible to prevent carcinogenesis by the excision of the precursor lesions. Ki-67 expression, both in the crypt and surface epithelium, and cyclin D1, c-erb B2 and bcl-2 over-expression may be a clue of dysplastic epithelium. This can be helpful in the differential diagnosis of regenerative atypia and flat adenoma. If the role of HPV is clarified and demonstrated in colonic carcinogenesis, vaccination could prevent carcinogenesis caused by HPV. Of course, it is too early to be hopeful about vaccination but well designed studies would be useful.

ACKNOWLEDGEMENT

This study is approved by Baskent University Hospital ethics committee and supported by Baskent University research fund. Many thanks to Dr Coskun Bakar for the statistical analyses and Busra Okursoy for the immunohistochemistry, and for the *in-situ* hybridization method applications.

REFERENCES

1. Contu PC, Contu SS, Moreira LF. Bcl-2 Expression in rectal Cancer. Arq Gastroenterol, 2006; **43:** 24–287.
2. Hamilton SR, Aaltonen LA, eds. World Health Organization classification of tumours. Pathology and genetics of tumours of the digestive system. Lyon: IARC Press; 2000: 103−37.
3. Koornstra JJ, de Jong S, Hollema H, de Vries EGE, Kleibeuker JH. Changes in apoptosis during the development of colorectal cancer: a systematic review of the literature. Critical Reviews in Oncology/Haematology 2003; **45:** 37−53.
4. Robbins SL, Kumar V, Abbas AK, Cotran RS, Fausto N. Robbins and Cotran pathologic basis of disease. 8th ed. Philadelphia: Saunders Elsevier; 2010: 822−5.
5. Lorenzon L, Ferri M, Emanuella Pilozzi, Torrisi MR, Ziparo V, French D. Human papillomavirus and colorectal cancer: evidences and pitfalls of published literature. Int J Colorectal Dis 2011; **26:** 135−42.
6. Vogelstein B, Fearon ER, Hamilton SR, Kern ES, Preisinger AC, Leppert M et al. Genetic alterations during colorectal-tumour development. N Engl J Med 1988; **319:** 525−32.
7. Kouraklis G, Theocharis S, Vamvakas P, Vagianos C, Glinavou A, Giaginis C et al. Cyclin D1 and Rb protein expression and their correlation with prognosis in patients with colon cancer. World Journal of Surgical Oncology 2006; **4:** 5.
8. Bukholm IK, Nesland JM. Protein Expression of p53, p21 (WAF1/CIP1), bcl-2, Bax, Cyclin D1and pRb in human colon carcinomas. Virchow's Arch 2000; **436:** 224−8.
9. Andersen SN, Rognum TO, Baka A, Clausen OPF. Ki-67: A useful marker for the evaluation of dysplasia in ulcerative colitis. J Clin Mol Pathol 1998; **51:** 327−32.
10. Tavangar SM, Shariftabrizi A, Soroush AR. Her/neu over-expression correlates with more advanced disease in Iranian colorectal cancer patients. Med Sci Monit 2005; **11:** CR123−6.
11. Lai M, Luo M, Yao J, Chen P. Anal cancer in Chinese: human papillomavirus infection and altered expression of p53. World J Gastroenterol 1998; **4:** 298−302.
12. Ryan DP, Compton CC, Mayer RJ. Carcinoma of the anal canal. New Engl J Med 2000; **342:** 792–800.
13. Zhang J, Ding Y, Zhou Z, Li H, Zhou. Expression of human papillomavirus 16 E7 DNA in patients with colorectal adenocarcinoma. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi 2005; **22:** 1024−6, 1044.
14. Perez LO, Abba MC, Laguens RM, Golijow CD. Analysis of the colon and rectum: detection of human papillomavirus (HPV) DNA by polymerase chain reaction. Colorectal Dis 2005; **7:** 492−5.
15. Buyru N, Tezol A, Dalay N. P53 intronic G13964C variant in colon cancer and its association with HPV. Anticancer Res 2005; **25:** 2767−9.
16. Buyru N, Budak M, Yazici H, Dalay N. P53 gene mutations are rare in human papillomavirus-associated colon cancer. Oncol Rep 2003; **10:** 2089−92.
17. Zhu Q, Cao J, Li S. Detection of human papillomavirus gene in biopsies from colon carcinoma by PCR. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi 1999; **13:** 352−4.
18. Bodaghi S, Yamanegi K, Xiao SY, Da Costa M, Palefsky JM, Zheng ZM. Colorectal papillomavirus infection in patients with colorectal cancer. Clin Cancer Res 2005; **11:** 2862−7.
19. Liang JJ, Alrawi S, Tan D. Nomenclature, molecular genetics and clinical significance of the precursor lesions in the serrated polyp pathway of colorectal carcinoma. Int J Clin Exp Pathol 2008; **1:** 317−24.
20. Unal H, Selcuk H, Gokcan H, Tore E, Sar A, Korkmaz M et al. Malignancy risk of small polyps and related factors. Dig Dis Sci 2007: DOI 10.1007/s10620-007-9782-8.
21. Suzuki Y, Honma T, Hayashi S, Ajioka Y, Asakura H. Bcl-2 expression and frequency of apoptosis correlate with morphogenesis of colorectal neoplasia. J Clin Pathol 2002; **55:** 212−16.
22. Zavrides HN, Zizi-Sermpetzogluo A, Panousopoulos D, Athanasas G, Elemenoglou I, Peros G. Prognostic evaluation of CD44 expression in correlation with bcl-2 and p53 in colorectal cancer. Folia Histochemica et cytobiologica 2005; **43:** 31−6.
23. Saleh HA, Jackson H, Khatib G, Banerjee M. Correlation of bcl-2 oncoprotein immunohistochemical expression with proliferation index and histopathologic parameters in colorectal neoplasia. Pathology Oncology Research 1999; **5:** 273–9.
24. Sun X, Gong Y, Talamonti MS, Rao S. Expression of cell adhesion molecules, cd44s and e-cadherin, and microvessel density in carcinoid tumours. Mod Pathol 2002; **15:** 1333−8.
25. Hori H, Fujimori T, Fujii S, Ichikawa K, Ohkura Y, Tomita S et al. Evaluation of tumour cell dissociation as a predictive marker of lymph node metastasis in submucosal invasive colorectal carcinoma. Dis Colon Rectum 2005; **48**: 938–45.
26. Houston M. Chapter 20. Breast. In: Rosai J, ed. Rosai and Ackerman's Surgical Pathology. 9th edition, volume 2. St Louis: Mosby; 2004: 1819.
27. Alao JP. The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. Molecular Cancer 2007; **6:** 24.
28. Leedham SJ, Schier S, Thliveris AT, Halberg RB, Newton MA, Wright NA. From gene mutation to tumours-stem cells in gastrointestinal carcinogenesis. Cell Prolif 2005; **38:** 387−405.
29. Demirbaş S, Sücüllü İ, Yıldırım Ş, Çelenk T. Influence of c-er B-2, nm23, bcl-2 and p53 protein markers on colorectal cancer. Turk J Gastroenterol 2006; **17:** 13−19.

30. Bousserouel S, Lamy V, Gosse F, Lobstein A, Marescaux J, Raul F. Early modulation of gene expression used as a biomarker for chemoprevention in a preclinical model of colon carcinogenesis. Pathology International 2011; **61:** 80−7.
31. Wong NACS, Morris RG, McCondochie A, Bader S, Jodrell DI, Harrison DJ. Cyclin d1 over-expression in colorectal carcinoma *in vivo* is dependent on ß-catenin protein dysregulation, but not k-ras mutation. J Pathol 2002; **197:** 128−35.
32. Wang L, Cao X, Chen Q, Zhu T, Zhu H, Zheng L. DIXDC1 targets p21 and cyclin D1 via PI3K pathway activation to promote colon cancer cell proliferation. Cancer Sci 2009; **100:** 1801−8.
33. Liang J, Pan Y, Zhang D. Cellular prion protein promotes proliferation and G1/S transition of human gastric cancer cells SGC7901 and AGS. Faseb J 2007; **21:** 2247−56.
34. Sun N, Meng Q, Tian A. Expression of the anti-apoptotic genes Bag-1 and Bcl-2 in colon cancer and their relationship. Am J Surg 2010; **200:** 341−5.
35. Kruszewski W, Kowara R, Rzepko R, Warezak C, Zielinski J, Gryglewski G et al. K-RAS point mutation, and amplification of c-myc and c-erb b2 in colon adenocarcinoma. Folia Histochemica et Cytobiologica 2004; **42:** 173−9.
36. Furuta K, Yoshioka S, Okabe S, Ikeda M, Oginosawa M, Ikeda S et al. Expressions of two adenomatous poliposis coli and e-cadherin proteins on human colorectal cancers. Virchow's Arch 2003; **442:** 266−70.
37. Hori H, Fujimori T, Fujii S, Ichikawa, Ohkura Y, Tomita S et al. Evaluation of tumour cell dissociation as a predictive marker of lymph node metastasis in submucosal invasive colorectal carcinoma. Dis Colon Rectum 2005; **48:** 938−45.
38. Greco C, Bralet MP, Ailane N, Dubart-Kupperschmitt A, Rubinstein E, Le Naour F et al. E-cadherin/p120-catenin and tetraspanin Co-029 cooperate for cell motility control in human colon carcinoma. Cancer Res 2010; **70:** 7674−83.
39. Cheng JY, Sheu LF, Lin JC, Meng CL. Detection of human papillomavirus DNA in colorectal adenomas. Arch Surg 1995; **130:** 73−6.
40. Breitburd F, Coursaget P. Human papillomavirus vaccines. Cancer Biology 1999; **9:** 431−5.
41. Eiben GL, Da Silva DM, Fausch SC, Le Poole IC, Nishimura MI, Kast WM. Cervical cancer vaccines: Recent advances in HPV research. Viral Immunology 2003; **16:** 111−21.
42. Mammas IN, Sourvinos G, Zaravinos A, Spandiodos DA. Vaccination against human papilloma virus (HPV): Epidemiological evidence of HPV in non-genital cancers. Pathol. Oncol Res, 2010; **17:** 103–19.
43. Damin DC, Caetano MB, Rosito MA. Evidence for an association of human papilloma virus infection and colorectal cancer. Eur J Surg Oncol 2007; **33:** 569−74.
44. Lu DW, El-Mofty SK, Wang HL. Expression of p16, Rb, and P53 proteins in squamous cell carcinomas of the anorectal cancer. Clin Cancer Res 2003; **11:** 2862−7.
45. Lee YM, Leu SY, Chiang H. Human papillomavirus type 18 in colorectal cancer. J Microbiol Immunol Infect 2001; **34:** 87−91.