

The potential role of the NEK6, AURKA, AURKB, and PAK1 genes in adenomatous colorectal polyps and colorectal adenocarcinoma

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Abstract Colorectal adenomatous polyp (CRAP) is a major risk factor for the development of sporadic colorectal cancer (CRC). Histone modifications are one of the epigenetic mechanisms that may have key roles in the carcinogenesis of CRC. The objective of the present study is to investigate the alterations in the defined histone modification gene expression profiles in patients with CRAP and CRC. Histone modification enzyme key gene expressions of the CRC, CRAP, and control groups were evaluated and compared using the reverse transcription PCR (RT-PCR) array method. Gene expression analysis was performed in the CRAP group after dividing the patients into subgroups according to the polyp diameter, pathological results, and morphological parameters which are risk factors for developing CRC in patients with CRAP. PAK1, NEK6, AURKA, AURKB, HDAC1, and HDAC7 were significantly more overexpressed in CRC subjects compared to the controls ($p < 0.05$). PAK1, NEK6, AURKA, AURKB, and HDAC1 were significantly more overexpressed in the CRAP group compared to the controls ($p < 0.005$). There were no significant differences between the CRAP and CRC groups with regards to PAK1, NEK6, AURKA, or AURKB gene overexpression. PAK1, NEK6, AURKA, and AURKB were significantly in correlation with the polyp diameter as they

were more overexpressed in polyps with larger diameters. In conclusion, overexpressions of NEK6, AURKA, AURKB, and PAK1 genes can be used as predictive markers to decide the colonoscopic surveillance intervals after the polypectomy procedure especially in polyps with larger diameters.

Keywords Colorectal adenocarcinoma · Colorectal adenomatous polyp · AURKA · AURKB · NEK6 · PAK1 · Epigenetic · Histone modification

Introduction

Colorectal cancer (CRC) is one of the leading causes of morbidity and mortality since it accounts for 10 % of all cancers in adults and the fourth leading cause of cancer-related deaths [1]. Most CRCs progress from colorectal adenomatous polyps (CRAPs) [2]. CRAPs are benign tumors of columnar cells or the glandular tissue, and sporadic adenomatous polyps tend to progress to cancer with increasing degrees of dysplasia associated with progressive accrual of several genetic and epigenetic changes [2, 3]. High malignancy risk criteria in CRAPs include advanced age, number of polyps, polyp diameter, villous component, nuclear atypia frequency, sessility, family history, and dysplasia severity. Of the adenomas larger than 1 cm, involving villous components and with severe dysplasia, 3, 17, and 37 %, respectively, transform to cancer annually [2, 4].

Different pathways such as chromosomal instability and microsatellite instability pathway, β -catenin/Wnt, TGF β /SMAD, and RAF/RAS/MAPK intermediary pathways have been described in CRC carcinogenesis. K-RAS mutation, B-RAF mutation, 18q deletion, APC mutation, p53 mutation as well as genetic and epigenetic alternations like DNA CpG islet hypermethylation, non-coding RNA (miRNA)

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expressions and specific histone modifications are observed in these pathways [5–7]. Epigenetic changes that result in subsequent aberrant gene expression are known to have key roles as ultimate predictors in inflammation-induced CRC carcinogenesis. Recent studies indicate that histone modification genes including NEK6, AURKA, PAK1, HDAC1, HDAC2, HDAC3, HDAC5, and HDAC7 have key roles in the carcinogenesis of CRC [8–16].

There is a clear need for more effective and selective markers to determine in CRAP patients who are at a higher risk of developing CRC. It is reasonable to commend that epigenetic alternations including histone modification have significant roles in cancer formation from CRAP tissues, and further studies are needed to elucidate this pathogenesis. There are no studies about the relationship comparing epigenetic chromatin histone modification patterns of CRAP and CRC in the literature. Therefore, we aimed in this study to investigate the value of chromatin histone modification in patient with regards to risk of CRC progression from CRAP.

Materials and methods

Study location The study was performed at the Gastroenterology and Medical Biology departments of Manisa Celal Bayar University Medical High School between November 2012 and November 2013. Patients presenting to the Gastroenterology Department of Manisa Celal Bayar University were included in the study.

Ethics committee This study was performed in accordance with the Declaration of Helsinki, good clinical practice, and applicable regulatory requirements. The study was carried out with the approval of the Institutional Ethical Review Board of the Celal Bayar University Medical Center with the approval number 269 dated 26 November 2012. Informed written consent form was obtained from each patient.

Study design and samples Fresh matched tissue samples were collected from a total of 75 patients presenting to the Gastroenterology Department of Celal Bayar University between November 2012 and November 2013 [group 1 (25 patients) was categorized as histopathologically confirmed CRC group; group 2 (25 patients) was categorized as the histopathologically confirmed CRAP group; group 3 (25 patients) was the control group and included patients with normal colon mucosa]. In addition, 25 patients in each group, 2 colon cancer samples, 3 polyp, and 1 control samples were lost due to RNA degradation. Patients were excluded from the study if they received colectomy for any reason, diagnosed with any types of malignancy, and had received chemotherapy or radiotherapy. Age, sex, and history/family history were

questioned and documented for all patients. All patients were checked for complete blood count and biochemical tests.

For patients with CRAP, polyp diameter, macroscopic polyp appearance (sessile/pedunculated), colonic location of the polyp, presence/number of any additional polyps at other foci as determined by colonoscopy, routine evaluations and examinations were documented for data purposes. Therapeutic polypectomy with endoscopic snare was performed for all polyps. All results of pathological assessments of the resected polyps such as histological type, morphological type (tubular, tubulovillous, villous), and presence of dysplasia were evaluated and documented.

For all patients diagnosed with CRC, CT or PET/CT was performed for malignancy staging and tumor type was identified in biopsy samples and colon resection material if the patient was operated, and the results were documented.

Colonoscopy Colonoscopic examinations of all patients were performed at the Gastroenterology Department of Celal Bayar University by the three gastroenterologists (EK, HY, EG) who performed the study. Olympus Luxera CFQ260AL was used for colonoscopy. Complete colonoscopic examinations including the terminal ileum were performed for all controls and diagnosed with CRAP. For patients with CRC, complete colonoscopic examination including the terminal ileum was performed if access to the proximal of the tumor could be achieved. If total ileocolonoscopy was not successful, proximal of the tumor was assessed with abdominal CT. For pathologic assessments, mucosal biopsy samples were collected from the tumor tissue in the CRC group, from the polyp tissue in the CRAP group, and from rectal, cecal, and terminal ileal mucosa in the control group using Olympus biopsy forceps. Pathologic assessments of all biopsy samples were performed in the Pathology Department of Celal Bayar University, and the resulting data were documented.

Additional mucosal biopsy samples from the tumor tissue of the CRC patients, from the polyp tissue of the CRAP patients, and from normal appearing mucosa of the control group were collected for histone modification gene expression analyses using Olympus biopsy forceps.

The tissue samples were immediately frozen using dry ice (a block of dry ice has a surface temperature of -78.5°C) and stored at -80°C until RNA extraction.

RNA isolation from tissue Total RNA was isolated using RNeasy Mini Kit (Qiagen, Germany) described in the manufacturer's protocol with small modifications. The fresh tissue samples (20–30 mg) from patients were with 600 μl Buffer RLT and a metal ball with a 7 mm diameter homogenized in TissueLyser II homogenizer (Qiagen, Germany) at 25,000 Hz

for 5 min. The lysate was centrifuged at maximum speed, and supernatant was treated after this point following the RNeasy Mini Kit protocol.

Quantity and purity of total RNA RNA was quantified measuring the absorbance at 260 nm (A260), and RNA purity was determined by the ratio A260/A280 using spectrophotometer. RNA quality was considered to be acceptable with A260/A280 ratio slightly higher than 2.0 and A260/A230 ratio slightly higher than 1.8. All steps were taken according to the manufacturer's protocol.

cDNA synthesis We performed the complementary DNA (cDNA) synthesis with RT First Strand Kit (C-03) (SA Bioscience, Frederick, MD, USA). Eight microliters of RNA sample was incubated with 2 μ l of GE (5 \times gDNA Elimination Buffer) at 42 $^{\circ}$ C for 5 min in a 0.2 ml PCR tube. In another tube, a PCR cocktail (4 μ l RT Buffer 3 (5 \times BC3), 1 μ l P2 (Primer and External Control mix), 2 μ l RT Enzyme Mix 3 (RE3), and 3 μ l H₂O) were prepared and added onto the RNA sample following by a 15-min incubation at 42 $^{\circ}$ C and 5-min incubation at 95 $^{\circ}$ C. The cDNA samples were later diluted as needed. All steps were taken according to the manufacturer's protocol.

Human Epigenetic Chromatin Modification Enzyme RT² Profiler™ PCR Array The Human Epigenetic Chromatin Modification Enzyme RT² Profiler™ PCR Array (PAHS-085A) (SA Biosciences, Frederick, MD, USA) was used to detect the expression levels of 84 key genes listed in Table 1.

Table 1 Histone modification genes to be investigated

DNA methyl transferase: DNMT1, DNMT3A, DNMT3B
Histone acetyl transferase: ATF2, CDYL, CIITA, CSRP2BP, ESCO1, ESCO2, HAT1, KAT2A (GCN5L2), KAT2B (PCAF), KAT5 (HTATIP), MYST1, MYST2, MYST3, MYST4, NCOA1, NCOA3, NCOA6
Histone methyl transferase: CARM1 (PRMT4), DOT1L, EHMT2, MLL1, MLL3, PRMT1, PRMT2, PRMT3, PRMT5, PRMT6, PRMT7, PRMT8, SETDB2, SMYD3, SUV39H1
SET domain proteins (activation of histone methyl transferase): ASH1L, MLL3, MLL5, NSD1, SETD1A, SETD1B, SETD2, SETD3, SETD4, SETD5, SETD6, SETD7, SETD8, SETDB1, SUV39H1, SUV420H1, WHSC1
Histone phosphorylation: AURKA, AURKB, AURKC, NEK6, PAK1, RPS6KA3, RPS6KA5
Histone ubiquitination: DZIP3, MYSM1, RNF2, RNF20, UBE2A, UBE2B, USP16, USP21, USP22
DNA/histone demethylase: AOF2, JARID1B, JARID1C, JMJD2A, JMJD2C, JMJD3, MBD2
Histone deacetylase: HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC6, HDAC7, HDAC8, HDAC9, HDAC10, HDAC11

A total of 2300 μ l reverse transcription PCR (RT-PCR) array mix (102 μ l diluted cDNA, 1150 μ l 2 \times RT² SYBR Green ROX FAST Master mix, and 1048 μ l H₂O) was prepared and loaded on 96-well Human Epigenetic Chromatin Modification Enzymes RT² Profiler™ PCR arrays 20 μ l per well. Arrays were placed in *Rotor-Gene* RG-3000 (Corbett Research, Qiagen, Germany) and were initially activated at 95 $^{\circ}$ C for 10 min followed by 40 cycles of 95 $^{\circ}$ C for 15 s and 60 $^{\circ}$ C for 30-s incubation. Array data was analyzed, and eight genes were determined to be the most overexpressed or underexpressed to cross-validate by RT qPCR.

RT² qPCR primer assay The cDNA samples of the eight genes chosen to be overexpressed or underexpressed (AURKB, AURKA, SETD8, PAK1, NEK6, KDM4C, HDAC1, HDAC7) and one housekeeping gene (HPRT1) were further tested by RT qPCR. The Primer Assays and SYBR Green Master Mix (SA Biosciences, Frederick, MD, USA) specific for the eight genes were purchased, and each PCR reaction mix was prepared by adding 12.5 μ l of SYBR Green Master Mix and 1 μ l of Primer (10 pmol) per 2.2 μ l cDNA sample. Total volume was adjusted to 25 μ l with dH₂O, and PCR mixes were loaded on the plate of *Rotor-Gene* RG-3000 (Corbett Research, Qiagen, Germany) for amplification. Amplification was performed with an initial denaturation step at 95 $^{\circ}$ C for 5 min followed by 40 cycles of 94 $^{\circ}$ C for 1 min, 61 $^{\circ}$ C for 40 s, and 72 $^{\circ}$ C for 1 min with a final elongation step at 72 $^{\circ}$ C for 2 min. Cycle threshold (Ct) values obtained from the RT qPCR reactions were normalized to the reference gene (HPRT1) and evaluated using Relative Expression Software Tool (REST) 2009 (V.2.0.13) in standard mode. All steps were taken according to the manufacturer's protocol.

Data analysis and statistics Data were analyzed using RT² profiler PCR array and RT² qPCR primer assay data analysis software (<http://www.sabiosciences.com/pcrarraydataanalysis.php>). The website allowed online analysis. PCR array was quantified based on the Ct number. A gene was considered to be not detectable when Ct is above 32. Ct was defined as 35 for the Δ Ct calculation when the signal was under detectable limits. Fold change and fold regulation values over 2 were indicative of upregulated genes; fold change values under 0.5 and fold regulation values under -2 were indicative of downregulated genes. Results were expressed as the mean values \pm standard deviation, and the *p* values were calculated based on a Student's *t* test of the replicate $2^{-\Delta$ Ct values for each gene in the control group, CRC group, and CRAP group. The characteristics data among the three groups were obtained using Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL 60606–6412) version 15.0 for Windows 7. The chi-squared test and Fisher's exact test were used to determine

among the three groups. Age was compared using the two-tailed *t* test. A *p* value under 0.05 was regarded as statistically significant.

Results

A total of 75 patients included in the study were divided into three groups: 25 patients in the CRC group, 25 patients in the CRAP group, and 25 subjects in the control group. The mean age of the patients in the study group was 59.49 ± 11.34 years. The mean age was 60.96 ± 12.18 years in the CRC group, 60.48 ± 10.31 years in the CRAP group, and 57.04 ± 10.60 in the control group. The three groups did not differ significantly with regards to age ($p > 0.05$). The proportion of male gender in the 75 subjects was 57.3 % ($n = 43$). In the CRC, CRAP, and control groups, 40 % ($n = 10$), 68 % ($n = 17$), and 64 % ($n = 16$) were males, respectively. (CRC M/F 10/15, CRAP M/F 17/8, control M/F 16/9). The characteristics of the three groups were summarized in Table 2. No significant difference was observed among the biochemical data of AURKB, AURKA, SETD8, PAK1, NEK6, KDM4C, HDAC1, and HDAC7 genes in all three groups, $p > 0.05$.

Colorectal cancers and gene expression analysis

In CRC patients ($n = 25$), AURKA, AURKB, HDAC1, HDAC7, NEK6, and PAK1 genes were overexpressed (fold change > 2) (Figs. 1 and 2), and the overexpression of the AURKA, AURKB, HDAC1, HDAC7, NEK6, and PAK1 genes was statistically significantly higher levels than the control group ($p < 0.05$) (Table 3) (Fig. 2). Sites of involvement of CRCs were assessed, and the majority of the tumors were located in the distal colon. The proportion of CRC patients by site of involvement were as follows: rectum 40 % ($n = 10$), sigmoid colon 28 % ($n = 7$), descending colon 4 % ($n = 1$), transverse colon 12 % ($n = 3$), ascending colon 8 % ($n = 2$), and cecum 8 % ($n = 2$). Pathological assessment demonstrated adenocarcinoma for all CRC patients. Of the patients, 28 %

($n = 7$) had poorly differentiated, 64 % ($n = 16$) had moderately differentiated tumors, and only 8 % ($n = 2$) had well-differentiated tumors. AURKA, AURKB, NEK6, PAK1, HDAC1, and HDAC7 genes were statistically significantly more overexpressed in poorly differentiated tumors than well and moderately differentiated tumors in the CRC (Table 4). The pathological classification and staging of CRC was in accordance with the American Joint Committee on Cancer (AJCC) tumor-node-metastasis (TNM) staging system (seventh edition, 2010). Of the patients, 68 % ($n = 17$) were in stage III and IV and 32 % ($n = 17$) were in stage I and II. AURKA, AURKB, NEK6, PAK1, HDAC1, and HDAC7 genes were statistically significantly more overexpressed in stage TNM III–IV than stage I–II in the CRC (Table 5). Of the patients, 40 % ($n = 10$) had higher than 5 ng/ml carcinoembryonic antigen (CEA) and 60 % ($n = 15$) lower than 5 ng/ml. There was no significant difference between higher than 5 ng/ml CEA and lower than 5 ng/ml CEA in the CRC group (Table 6).

Colorectal adenomatous polyps and gene expression analysis

The patients with polyps ($n = 25$) AURKA, AURKB, NEK6, PAK1, and HDAC1 genes were statistically significantly more overexpressed compared to the control group (fold change > 2 , $p < 0.05$). (Figs. 2 and 3 and Table 7). The comparison of the CRC and CRAP groups demonstrated that the HDAC1 and HDAC7 genes were statistically significantly more expressed in the former group ($p < 0.05$) (Table 8).

Most of the colorectal adenomatous polyps were located in the distal and left colon as a CRC. The proportions of patients with CRAP by the location of polyp were as follows: rectum 16 % ($n = 4$), sigmoid colon 36 % ($n = 9$), descending colon 24 % ($n = 6$), transverse colon 4 % ($n = 1$), and ascending colon 20 % ($n = 5$).

Gene expression analysis by pathological assessment The patients with polyps ($n = 25$) were divided into three subgroups by pathological assessments as villous (32 %, $n = 8$),

Table 2 Characteristics of the patient and control population

	CRC	CRAP	Control group	<i>p</i> Value
Age	60.96 ± 12.18	60.48 ± 10.31	57.04 ± 10.60	NS
Male	40 %	68 %	64 %	$< 0.05^*$
Hemoglobin (g/dl)	10,824	11,352	12,032	$< 0.05^{\#}$
Albumin (g/dl)	2752	2948	3096	$< 0.05^{\#}$
Leukocyte count (U/mm ³)	10,420	11,210	11,480	NS
Platelet count at admission (10 ³ U/mm ³)	183.5	210.3	205.3	NS

* $p < 0.05$, CRAP and control group were significantly higher than CRC group

[#] $p < 0.05$, CRC was significantly lower than control group

NS not significant

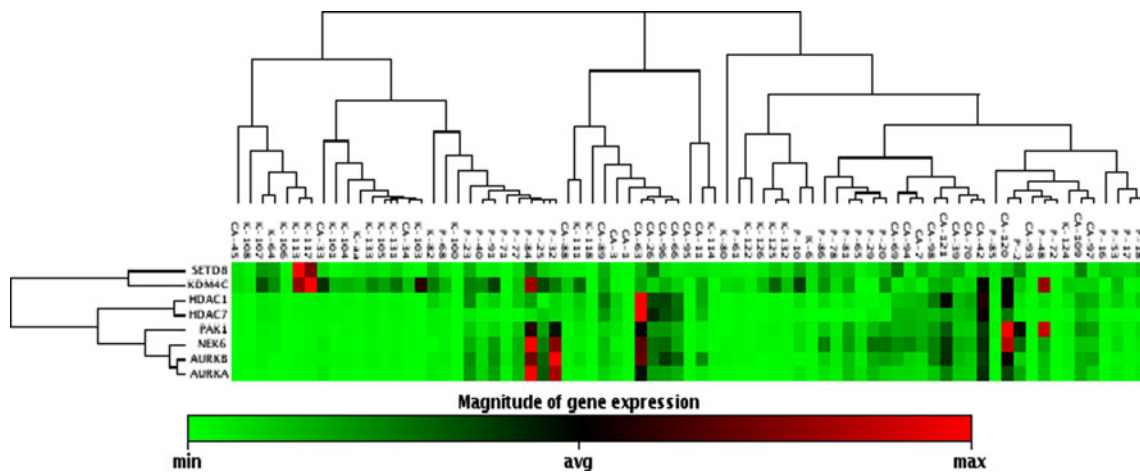


Fig. 1 The clustergram creates a heat map with dendrograms to indicate genes that are co-regulated. The color saturation reflects the magnitude of the change in gene expression. *Green squares* represent lower gene expression in the experimental samples (ratios <1); *black squares* represent genes equally expressed (ratios near 1); *red squares* represent

higher than control levels of gene expression (ratios >1); *gray squares* indicate insufficient or missing data. The *x*-axis indicates the groups (CRC colorectal cancer, CRAP colorectal adenomatous polyp, CTR control group), and the *y*-axis indicates the genes

tubulovillous (40 %, *n*=10), and tubular (28 %, *n*=7) subgroups. Early onset adenocarcinoma foci or carcinoma and high-grade dysplasia were not observed in situ in any of the polyps.

The subgroups were compared both among themselves and to the control group for gene expressions. In the

villous adenoma subgroup, AURKA, AURKB, PAK1, NEK6, HDAC1, and HDAC7 genes were overexpressed (fold change>2). In the tubulovillous adenoma subgroup, AURKA, NEK6, and PAK1 genes were overexpressed (fold change>2). However, inter-comparison of the subgroups did not yield a statistically significant difference (Tables 9 and 10).

Fig. 2 (*Overexpressed genes in CRC*): Hybridization intensity of each gene in the CRC and control groups appears as a log 10 base scattered plateau. The *x*-axis represents the control group, and the *y*-axis represents the CRC group

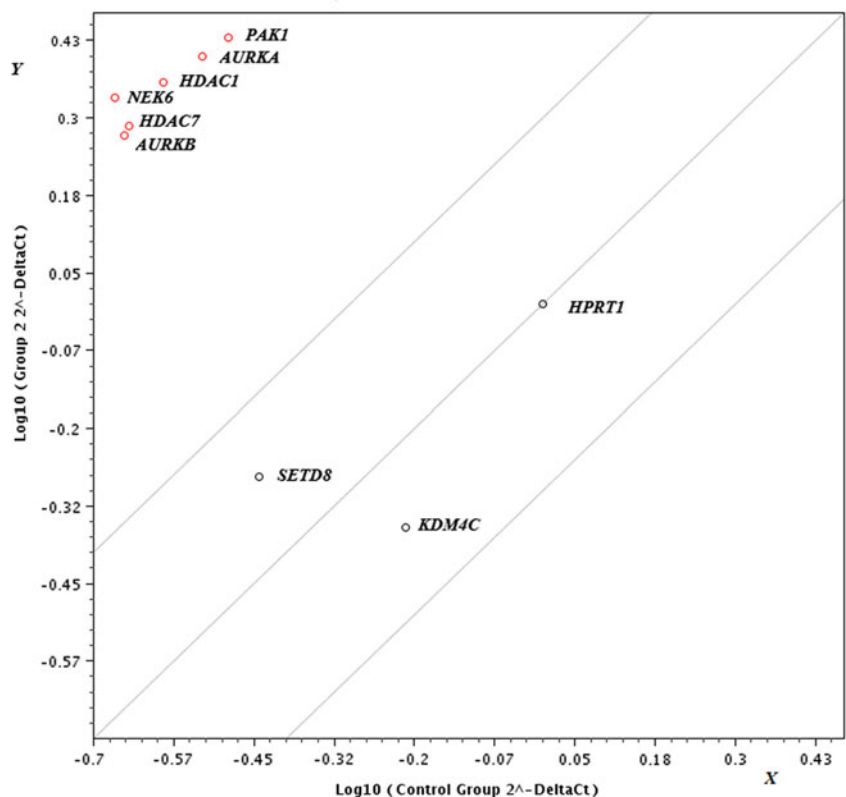


Table 3 Histone modification gene expression analysis in CRC

Histone gene	Fold change	95 % CI	<i>p</i> Value
AURKB	8.3675	(2.67, 14.07)	0.000032
AURKA	8.5079	(2.78, 14.23)	0.000095
SETD8	1.4564	(0.55, 2.36)	0.608738
PAK1	8.312	(2.15, 14.47)	0.000728
NEK6	9.9728	(2.78, 17.16)	0.000134
KDM4C	0.716	(0.27, 1.16)	0.127516
HDAC1	8.8716	(2.73, 15.01)	0.000107
HDAC7	8.5457	(2.78, 14.31)	0.000408
HPRT1	0.9516	(0.73, 1.18)	0.797837

CRC group was compared with the control group

The bold ones are significant results

Gene expression analysis by polyp diameter The largest polyp diameter was 40 mm, and the smallest was 7 mm, and the mean polyp diameter was 16.72±9.56 mm. The patients were divided into two subgroups by polyp diameter as ≥10 mm polyps (64 %, *n*=16) and <10 mm polyps (36 %, *n*=9). The extent of overexpression of AURKA, AURKB, PAK1, and NEK-6 and HDAC1 genes was statistically significantly greater in patients with polyp diameters ≥10 mm compared to those with polyp diameters <10 mm (Table 11).

Gene expression analysis by polyp morphological appearance The patients were divided into two subgroups by macroscopic polyp classification with colonoscopy as sessile polyps (28 %, *n*=7) and pedunculated polyps (72 %, *n*=18). Patients with sessile polyps and pedunculated polyps did not differ statistically significantly (Table 12).

Table 4 Histone modification gene expression analysis in CRC according to tumor differentiation

Histone gene	Fold change	95 % CI	<i>p</i> Value
AURKB	4.9692	(0.81, 9.12)	0.009603
AURKA	4.2114	(0.25, 8.17)	0.035442
SETD8	2.1716	(0.75, 3.59)	0.052253
PAK1	3.6309	(0.00001, 7.69)	0.033249
NEK6	3.5612	(0.00001, 7.59)	0.048957
KDM4C	2.083	(0.67, 3.49)	0.302539
HDAC1	4.4775	(0.89, 8.06)	0.014735
HDAC7	4.7378	(0.74, 8.74)	0.013279
HPRT1	1	(1.00, 1.00)	–

Poorly differentiated tumors was compared with the well and moderately differentiated tumors in the CRC group

The bold ones are significant results

Table 5 Histone modification gene expression analysis in CRC according to TNM staging

Histone gene	Fold change	95 % CI	<i>p</i> Value
AURKB	9.791	(0.26, 19.33)	0.005209
AURKA	8.8907	(0.00001, 18.25)	0.013707
SETD8	2.1213	(0.58, 3.67)	0.130966
PAK1	9.1852	(0.00001, 18.71)	0.038042
NEK6	10.2353	(0.00001, 21.08)	0.01096
KDM4C	1.7171	(0.15, 3.28)	0.46629
HDAC1	8.6822	(0.99, 16.37)	0.007836
HDAC7	10.0073	(1.84, 18.18)	0.009863
HPRT1	1	(1.00, 1.00)	–

TNM III–IV was compared with the TNM I–II in the CRC group

The bold ones are significant results

Discussion

Understanding of the epigenetic changes in cancer cells leads to important information on the stages of carcinogenesis [17]. Monitoring of precancerous and cancerous cells improves as our limited body of knowledge on cancer molecular biology expands [18, 19]. The fact that epigenetic alterations, in contrast to genetic ones, are reversible support, the concept of those epigenetic treatments may be used in the future [20].

PAK1 (p-21 activated kinase) coordinates RAS/RAF/MAPK and Wnt/β-catenin signaling in CRC by acting small GTPases [21–23]. There are no studies in the literature demonstrating PAK1 gene overexpression in the polyp tissue, which is a precancerous tissue for CRC.

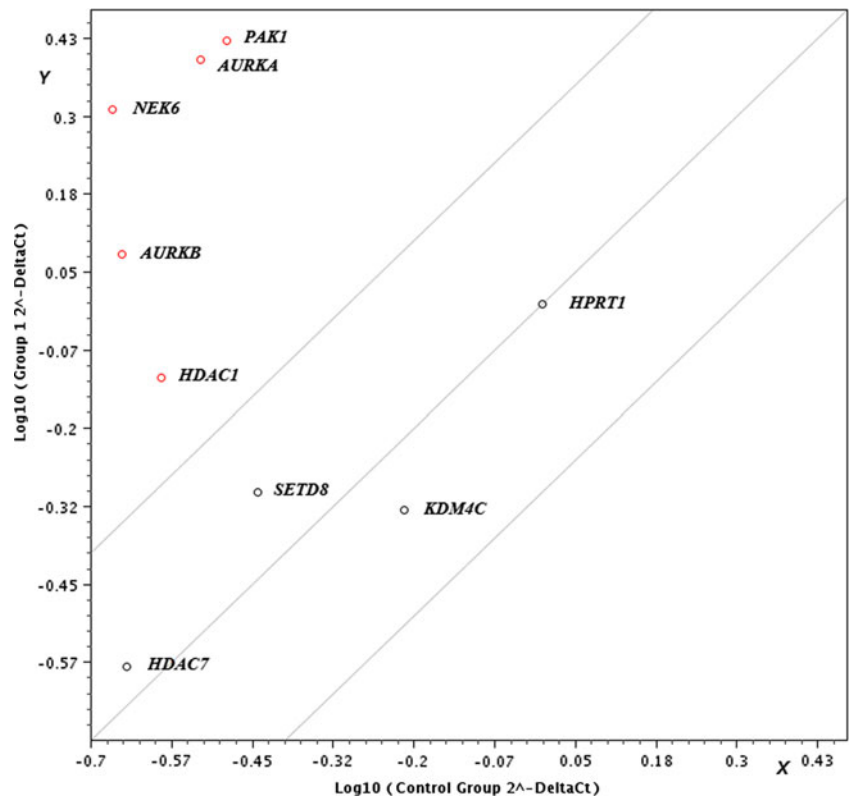
The aurora kinase family (AURKA, AURKB, AURKC) is serine/threonine kinases involved as key regulators in mitosis and regulate the G2/M phase of the cell cycle. AURKA mRNA amplification has been established in many cancers including CRC [24–26]. A study described that high

Table 6 Histone modification gene expression analysis in CRC according to CEA

Histone gene	Fold change	95 % CI	<i>p</i> Value
AURKB	1.2976	(0.00001, 3.08)	0.3789
AURKA	1.2958	(0.00001, 3.02)	0.564171
SETD8	0.9624	(0.25, 1.67)	0.915869
PAK1	1.1394	(0.00001, 2.74)	0.905578
NEK6	1.256	(0.00001, 3.06)	0.689286
KDM4C	0.8699	(0.21, 1.53)	0.347131
HDAC1	1.0961	(0.00001, 2.54)	0.410551
HDAC7	1.1512	(0.00001, 2.64)	0.445604
HPRT1	1	(1.00, 1.00)	–

CEA >5 ng/dl was compared with the CEA <5 ng/dl in the CRC group

Fig. 3 (*Overexpressed genes in CRAP*): Hybridization intensity of each gene in the colorectal adenomatous polyp (CRAP) and control groups appears as a log 10 base scattered plateau. The x-axis represents the control group, and the y-axis represents the CRAP group



AURKA expression was associated with increased recurrence/relapse frequency [12]. NEK6 is a serine/threonine kinase from the never in mitosis gene a (NIMA) [27]. Studies have observed termination of mitotic division, chromatin spindle defects, abnormal chromosome differentiation, and apoptosis induction with NEK6 dysfunction [28–30]. There are no studies in the literature demonstrating NEK6, AURKA, and AURKB overexpression in the polyp tissue, which is a precancerous tissue for CRC. NEK6 and AURKA are overexpressed in esophagitis [31], and AURKA is overexpressed in Barrett’s esophagus [32]. A

study by Katsha et al. found higher levels of AURKA in premalignant and malignant lesions compared to healthy gastric tissue [33]. There are studies which suggested that AURKA inhibitors could be developed as therapeutic agents for gastric cancer [33]. Nassirpour et al. have shown that the kinase activity of NEK6 was found to be increased in several cancer cells including colorectal cancer and that suppression of NEK6 resulted in tumor regression in a mice xenograft model [16].

HDACs are a critical family of proteins involved in the transcriptional regulation of a large number of genes and in

Table 7 Histone modification gene expression analysis in CRAP

Histone gene	Fold change	95 % CI	p Value
AURKB	5.3636	(1.57, 9.16)	0.006183
AURKA	8.3374	(2.51, 14.16)	0.006409
SETD8	1.3778	(0.49, 2.27)	0.559296
PAK1	8.1319	(2.76, 13.50)	0.002055
NEK6	9.4926	(3.00, 15.98)	0.000946
KDM4C	0.764	(0.21, 1.32)	0.677434
HDAC1	2.9567	(1.19, 4.73)	0.004393
HDAC7	1.1564	(0.49, 1.82)	0.433832
HPRT1	1.0134	(0.78, 1.24)	0.888077

The patient with polyp was compared with the control group
The bold ones are significant results

Table 8 Comparison of CRC and CRAP groups for gene expressions

Histone gene	Fold change	95 % CI	p Value
AURKB	1.5601	(0.23, 2.89)	0.565911
AURKA	1.2203	(0.21, 2.23)	0.92595
SETD8	1.057	(0.50, 1.61)	0.875204
PAK1	1.0221	(0.22, 1.82)	0.81793
NEK6	1.0506	(0.14, 1.96)	0.841459
KDM4C	0.9372	(0.30, 1.57)	0.256543
HDAC1	3.0005	(0.93, 5.08)	0.001429
HDAC7	7.3901	(2.21, 12.57)	0.00058
HPRT1	0.939	(0.72, 1.16)	0.701363

CRC patient group was compared with CRAP patient group
The bold ones are significant results

Table 9 Gene expression analysis by pathological assessment in CRAP

Histone gene	Fold change	95 % CI	<i>p</i> Value
AURKB	8.0467	(0.00001, 18.50)	0.084274
AURKA	9.0418	(0.00001, 21.54)	0.114475
SETD8	1.3042	(0.42, 2.19)	0.340087
PAK1	4.2866	(0.03, 8.54)	0.068487
NEK6	9.8626	(0.00001, 22.26)	0.05811
KDM4C	3.0269	(0.00001, 6.88)	0.120283
HDAC1	2.0072	(0.19, 3.82)	0.135356
HDAC7	1.3954	(0.00001, 3.14)	0.729216
HPRT1	1	(1.00, 1.00)	–

Villous adenoma subgroup was compared to tubular adenoma subgroup
The bold ones are significant results

the functional regulation of multiple proteins. Several studies have also examined HDAC expression in colon tumors. Most of these studies have reported increased expression of the HDAC1 and HDAC7 [11, 34]. Our findings are consistent with previous data in the villous adenoma subgroup and CRC patients that HDAC1 and HDAC7 were overexpressed (fold change > 2) (Figs. 1 and 2). But, HDAC1 and HDAC7 were not significantly different in other pathologic subgroups. Therefore, they may not be appropriate genetic markers for the early diagnosis of patients.

Current research suggests that PAK1, AURKA, NEK6, HDAC1, HDAC2, HDAC3, HDAC5, and HDAC7 genes play a key role in colorectal cancer carcinogenesis, and these genes is also an important prognostic and diagnostic marker in colorectal cancer and [8, 9, 12, 15, 16]. AURKA, AURKB, NEK6, PAK1, HDAC1, and HDAC7 was overexpressed in our study with poorly differentiated and advanced TNM. In light of the results, we speculate that these genes could be used as a prognostic marker in advanced CRC.

Table 10 Gene expression analysis by pathological assessment in CRAP

Histone gene	Fold change	95 % CI	<i>p</i> Value
AURKB	1.7902	(0.00001, 4.27)	0.715866
AURKA	2.7224	(0.00001, 6.55)	0.640497
SETD8	0.7218	(0.04, 1.40)	0.760542
PAK1	2.4925	(0.00001, 5.29)	0.223348
NEK6	3.9606	(0.00001, 9.12)	0.1095
KDM4C	1.6945	(0.00001, 3.90)	0.313541
HDAC1	1.1184	(0.04, 2.20)	0.390174
HDAC7	0.92	(0.00001, 1.85)	0.495245
HPRT1	1	(1.00, 1.00)	–

Tubulovillous adenoma subgroup was compared to tubular adenoma subgroup

The bold ones are significant results

Table 11 Gene expression analysis by polyp diameter in CRAP

Histone gene	Fold change	95 % CI	<i>p</i> Value
AURKB	11.144	(1.30, 20.98)	0.032898
AURKA	9.6339	(0.27, 19.00)	0.047747
SETD8	1.9452	(0.16, 3.73)	0.283834
PAK1	5.4916	(1.49, 9.50)	0.027911
NEK6	9.5214	(1.12, 17.93)	0.01425
KDM4C	3.0311	(0.00001, 6.30)	0.08482
HDAC1	1.7761	(0.10, 3.45)	0.279395
HDAC7	2.4188	(0.61, 4.23)	0.060067
HPRT1	1	(1.00, 1.00)	–

Patients with poly diameter ≥ 10 mm were compared to patients with polyp diameter < 10 mm

The bold ones are significant results

This is the first study which shows that PAK1, NEK6, AURKA, and AURKB genes are significantly overexpressed in polyp tissue. Our study was able to arrive at this conclusion despite the presence of major dysplasia only in two of the subjects with polyps. In light of the results reported from the studies mentioned above and those obtained in our study, we believe that these four genes have significant involvements in the stages of CRC progressing from polyps, and we further believe that these four histone modification enzyme genes can be used in anticancer treatments as therapeutic targets.

In adenomatous polyps, the risk of developing CRC is known to increase with increasing age, number of polyps, polyp diameter, villous component ratio, nuclear atypia frequency, increased dysplasia severity and sessile polyp presence, and familial history of CRC [2, 4]. The better understanding of these risk factors [2, 4] and their integration to clinical practice results in significant reductions in morbidity and mortality [35]. Our study demonstrated a significant relationship between PAK1, NEK6, AURKA, and AURKB gene

Table 12 Gene expression analysis by morphological polyp appearance

Histone gene	Fold change	95 % CI	<i>p</i> Value
AURKB	0.5407	(0.00001, 1.44)	0.51232
AURKA	1.0799	(0.00001, 2.63)	0.646558
SETD8	1.6562	(0.61, 2.70)	0.661301
PAK1	0.9116	(0.00001, 1.87)	0.648576
NEK6	0.9149	(0.00001, 2.08)	0.977741
KDM4C	1.3023	(0.00001, 2.85)	0.946359
HDAC1	1.4609	(0.29, 2.63)	0.931662
HDAC7	1.5571	(0.00001, 3.23)	0.130108
HPRT1	1	(1.00, 1.00)	–

Sessile polyp subgroup was compared to pedunculated polyp subgroup

overexpressions and increased polyp diameter, which is a risk factor for CRC cancer development in polyp tissue. Based on this result, we may speculate that colonoscopic screening recommended by several guidelines following polypectomy for polyps larger than 10 mm may be performed at shorter intervals if these markers are tested positive. The extent of overexpression of the PAK1, NEK6, AURKA, and AURKB genes increase with the presence and progression of villous component in the polyp, which is another risk factor, although a statistically significant relationship could not be shown in the subgroups. Significant reductions will be achieved in morbidity and mortality if genetic and epigenetic markers, which may be used in CRC screening of patients with CRAP, are integrated to clinical practice.

In conclusion, we demonstrated the overexpression of PAK1, NEK6, AURKA, and AURKB genes in patients with CRAP and CRC in the Turkish population. Elucidation of the molecular pathophysiology of CRC and CRAP will contribute to the treatment and prognosis of the diseases. Further studies are needed to understand the roles of these four genes in the CRC carcinogenesis. In addition, PAK1, NEK6, AURKA, and AURKB genes may be studied as promising genetic markers in new studies in different populations.

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Compliance with ethical standards

Conflicts of interest None.

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