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RESEARCH

# Characteristics of Advanced- and Non Advanced Sporadic Polypoid Colorectal Adenomas: Correlation to KRAS Mutations

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**Abstract** The malignant potential of colorectal adenomas highly correlates with their pathological characteristics, such as size, histology and grade of dysplasia. Currently, based on these parameters, adenomas are characterized as "non-advanced or advanced" and patient surveillance is adjusted accordingly. The aim of this study was to investigate the correlation between the KRAS mutations and characteristics of non-advanced and advanced colorectal adenomas for predicting the risk of increased malignant potential of adenomas that may influence the decision to offer follow-up endoscopic surveillance. We used a mutagenic polymerase chain reaction – restriction fragment length polymorphism method to determine KRAS mutations in 164 colorectal sporadic polypoid adenomas (51 non-advanced-, 113 advanced adenomas) and in 40 early colorectal carcinomas. The method of mutation detection was validated according to recommendation for KRAS mutation testing in colorectal carcinoma of the European Quality Assurance Program. The limit of detection of the assay was 3 % mutated DNA with a good reproducibility. Evaluation of pathological characteristics was performed according to European Guidelines for Quality Assurance in Colorectal Cancer Screening and Diagnosis. The morphological parameters of the adenoma such as size, histology, grade of dysplasia are highly correlated with one another: an increasing adenoma size raised the proportion of villous histology and degree of dysplasia (all $p<0.0001$). KRAS mutations were detected in 31 % of the non-advanced adenomas, in 57.5 % of the advanced adenomas and in 62.5 % of the early carcinomas. Most mutations occurred at codon 12 rather than at codon 13 (72 %, 82 %, 76 % versus 22 %, 17 %, 24 %, respectively). There was no significant difference in association of KRAS mutation with age, gender, location among non-advanced-, and advanced adenomas and early carcinomas. KRAS mutation was found more often in tubulovillous and villous adenomas, whereas wild-type KRAS was observed more frequently in tubular adenomas ($P<0.0001$) and there was an increased prevalence of KRAS mutations in larger adenomas ($P<0.0001$). In this study KRAS mutation occurred with the same frequency in adenomas with low-grade (48 %) and high-grade (50 %) dysplasia. KRAS mutation is very strongly associated with a villous architecture and through villous component expansion, KRAS mutations may increase risk of tumor progression in sporadic colorectal polypoid adenomas.

**Keywords** Sporadic colorectal adenomas · Villous architecture · KRAS mutation · Adenoma progression

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## Introduction

Colorectal cancer (CRC) is the second most common malignancy in the western world and the incidence and mortality rates of CRC are on the rise, globally [2, 11, 14, 16]. It has been generally accepted that a majority of CRC develop from sporadic colorectal adenoma, through a well-defined adenoma-carcinoma sequence [9, 12] and colorectal

adenomas are fairly common in developed countries, approximately, 40–50 % of the population develop one or more adenomas in their lifetime, but only 5–6 % progress to malignant tumor [1, 3, 4, 6]. The risk of cancer developing within an adenoma increases with size, grade of dysplasia (synonymous with intraepithelial or mucosal neoplasia), and villosity. Adenomas 10 mm or larger in size or with high-grade dysplasia or those of villous structure are likely to contain carcinomas. Such adenomas are called "advanced adenomas" [5, 12–14, 29, 30]. However, every adenoma has the capacity of malignant transformation. Small size adenomas (less than <10 mm in size) represent an appreciable majority of colon adenomas and many of these adenomas have advanced histological features [16, 22]. Moreover, the endoscopic removal of adenomas reduces the incidence of CRC by as much as 90 %, by stopping the progression of precursor lesions to cancer [12]. The future risk of diagnosing cancer or advanced adenomas following adenoma removal depends on the characteristics of previously removed adenomas [14]. The accurate identification of advanced neoplasia of the removed lesions is therefore important for developing an appropriate surveillance strategy. Establishing a risk profile on the basis of the traditional features of adenoma such as larger adenoma size, grade of dysplasia and villosity is not always straightforward. Many authors still address the need for more objective, reproducible and standardized pathologic criteria and the identification of additional molecular profiles associated with characteristics of advanced adenomas will likely provide valuable information regarding the development prevention strategies [28–32].

KRAS gene is known to play an important role in the colorectal carcinogenesis and mutations in the KRAS gene develop early in the progression from adenoma to carcinoma and are responsible for the transformation and development of CRC [9, 10]. KRAS mutation has been strongly associated with advanced adenoma features [7, 8]. Individuals with colorectal adenoma >20 mm in size or bearing KRAS mutations were reported to have a higher risk of producing recurrent adenoma during the follow-up period [33], therefore the mutation of KRAS oncogene is regarded to be a critical step in colorectal tumorigenesis and could be used as objective evidence of aggression.

The aim of this study was to investigate those pathological features of non-advanced and advanced colorectal adenomas, which show overlapping between adenomas and early carcinomas in correlation to KRAS mutation. We hypothesized that the association of KRAS mutation with pathological variables of adenomas provides useful information to predict the potential propensity of colorectal tumors to transform into progressive condition and may influence the decision to offer follow-up endoscopic surveillance.

## Materials and Methods

### Patient Samples

A retrospective series of 164 colorectal sporadic polypoid adenomas from 148 patients (92 male, 56 female) and 40 early colorectal carcinomas (pT1 adenocarcinoma according to TNM), arose within adenoma were obtained from Pécs Medical University and Balassa Janos Hospital of Tolna County Municipality, Hungary. Samples resected colonoscopically and surgically between 2001 and 2010 were investigated. Clinical data were reviewed to ensure that all cases were indeed sporadic adenomas and carcinomas. On account of the low frequency of villous, tubulovillous and high-grade adenomas in population-based series of colorectal adenomas, the series therefore was selectively enriched for villous, tubulovillous and high-grade adenomas. Colorectal adenomas and carcinomas were classified according to anatomical site as follows. Proximal colon: cecum through transverse colon; distal colon: splenic flexure through sigmoid colon; rectum: rectosigmoid junction, and rectum. No information on location was present in the pathology and endoscopy reports of 2 adenomas and of 3 early carcinomas, so they were excluded from the statistical analysis. The clinicopathological characteristics of colorectal neoplasms are presented in Table 1.

### Morphologic Evaluation of Colorectal Neoplasms

Evaluation of colorectal neoplasia was performed according to European Guidelines (EUG) for quality assurance in colorectal cancer screening and diagnosis [11]. The size of adenomas was assessed by measuring the maximum diameter of the adenomatous component in millimeters (mm) on the haematoxylin-eosin (HE) stained slides or on the formalin-fixed specimen when the largest dimension of the lesion

**Table 1** Clinicopathological characteristics

| | |
|---|---|
| Adenoma | |
| Median age, years (range) | 67.0 (33–89) |
| Sex (male/female) | 92/56 |
| Location of adenoma (proximal/distal/rectum/unknown) | 23/59/80/2 |
| Median size of adenoma, mm (range) | 16.4 (2–80) |
| Histology of adenoma (tubular/tubulovillous/villous) | 35/86/43 |
| Grade of mucosal neoplasia (low/high) | 62/102 |
| Non-advanced/advanced adenoma | 51/113 |
| Early carcinoma | |
| Median age, years (range) | 66.8 (48–89) |
| Sex (male/female) | 27/13 |
| Location of carcinoma (proximal/distal/rectum/unknown) | 6/9/22/3 |

cannot be reliably measured if the lesion was too large on the slide. In the EUG, a classification system for colorectal neoplasia has been recommended based on a modified version of the revised Vienna classification and recommends a two-tiered grading of mucosal neoplasia. According to it, a neoplastic mucosal lesion was graded into low and high grade (for criteria see Table 2), the diagnostic grade was based on the most severely dysplastic area, independently of its extention. Early colorectal carcinoma was defined as showing carcinoma invasion through the muscularis mucosae into the submucosa but not into the muscularis propria.

Adenomas are divided into tubular, tubulovillous, villous types and demarcation between the three is based on the relative proportion of tubular and villous components, according to the "20 % rule" described in the WHO classification of tumors in the digestive tract [15]. Tubular adenoma was composed of villous component up to 20 % within the tumor, tubulovillous adenomas had a villous component of 21 % to 80 %, whereas a villous adenoma had a villous component of 81 % to 100 %.

HE stained slides were evaluated by two different pathologists independently in a blind manner, according to EUG criteria. In case of interobserver disagreement on the interpretation of histology, a consensus diagnosis was reached at a multi-headed microscope. Adenomas were sorted into two groups on the basis of their morphological characteristics. Advanced adenoma was defined as ≥10 mm in size or/and contains a high-grade mucosal neoplasia or/and a villous component. These features predict an increased likelihood of malignant transformation. Adenomas not fulfilling the criteria above were classified as non-advanced adenomas.

## KRAS Mutation Analysis

Genomic DNA was extracted from macrodissected sections of archival, paraffin-embedded tissues using a QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. KRAS mutation analysis at codon 12 and 13 was performed using mutagenic polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) assay. PCR –RFLP assay was validated according to Recommendation for KRAS mutation testing in colorectal carcinoma of the European quality assurance program [17]. Three different cell lines were used to evaluate the sensitivity of the mutation test: 1.K562, wild-type KRAS cell line [18]; 2. A549 cell line has a homozygous 34 G>A (G12S) mutation in codon 12; 3. HCT116 cell line has a heterozygous 38 G>A (G13D) mutation in codon 13 [19]. The detection limit of mutation detection was determined by using serial dilution of KRAS mutant in wild-type cells, ranging from 1 % to 100 %. Furthermore, DNA from cell lines K562, A549 and HCT116 were used as a negative and positive control.

## Mutagenic PCR-RFLP

We used mismatched upstream primers for codon 12 and 13 amplification. The primers used were as follows (Invitrogen Inc. USA): KRAS/12 (sense) 5′–GAATATAAA CTTGTGGTAGTTGGACCT-3′ and KRAS/13 (sense) 5′-TATAAACTTGTGGTAGTTGGCCCTGGT-3′ and KRAS/12/13 (antisense) 5′- GGTCCTGCACCAGTAATATG-3′. Reactions were performed in a volume of 25 μl containing 50–150 ng DNA sample, 10xPCR buffer, 3 mM $MgCL_2$, 200 μM of dNTPs, 0.1 μM of each primer for each codon, 0.75 U Ampli Taq Gold DNA Polymerase (Life Technologies, USA). After denaturation at 95 °C for 10 min the samples were subjected to 38 cycles of amplification. For codon 12, each cycle comprised 1 min denaturation at 95 °C, 1 min annealing at 55 °C, and 2 min elongation at 72 °C. The final cycle included an elongation step of 4 min at 72 °C. For codon 13, all steps were the same except for a 59 °C annealing temperature. The resulting amplified mixtures were run on 2 % agarose gels to confirm the presence of expected products. Each sample (15 μl) was then digested with the restriction enzymes BstNI (New England BioLabs) for codon 12 and BglI (New England BioLabs) for codon 13 at 60 °C and 37 °C

**Table 2** Grading of gastrointestinal neoplasia [13]

| | Normal | Low-grade mucosal/intraepithelial neoplasia (LGMN) | High-grade mucosal/intraepithelial neoplasia (HGMN) | Invasive carcinoma |
|---|---|---|---|---|
| Glands | Non-branching | Villous | Branching, cribriform, irregular, solid | Branching, irregular, cribriform, solid |
| Expansion | Up/down | Till surface | Till surface | Lateral expansion |
| Epithelial differentiation | Up/down | Top-down and exceptional down-top | No maturation towards surface | |
| Goblet cell | ++ | + | -/+ Retronuclear, atypic | |
| Nuclear rows | 1 | 2–3 | 2–5 | Changing |
| Nuclear size | Small, basal | Palisading | Enlarged | Vesicular |
| Chromatin | Few | + | ++ | ++/+++ |
| Nucleoli | None | None | Few small | Several/prominent |

respectively, for 4.16 h in a final volume of 30 μl. The products of the digestion were separated by electrophoresis on 3 % agarose gel, stained with ethidium bromide and photographed in UV light. We found coexisting KRAS mutations in codon 12 and 13, in two cases of the colorectal adenomas. Coexisting KRAS mutations of two cases were verified by direct sequencing, using the ABI 3100 Genetic Analyzer.

## Statistical Analysis

Statistical analysis was performed using the GraphPad PRISM® software. After proof of normality for data sets using both D'Agostino & Pearson omnibus and Shapiro-Wilk normality tests non-parametric ANOVA (Kruskal-Wallis) was performed to establish the difference in age when advanced, non-advanced and early cancer groups were compared at once. The age differences were assessed in two groups using unpaired t-tests where the two-tailed level of $p \leq 0.05$ was the significance threshold.

Chi square and Fisher exact test were used to evaluate the statistical significance of the relationships between explanatory variables such as gender, localization, and pathological features of non-advanced, advanced colorectal adenomas and early carcinomas. The null hypothesis was rejected for $p$ values less or equal than 0.05.

## Results

We analyzed 51 nonadvanced-, 113 advanced colorectal polypoid adenomas and 40 early colorectal adenocarcinomas, having developed within in adenoma. No significant difference was determined in age among patients with nonadvanced- and advanced adenomas and patients with early carcinomas using non-parametric ANOVA: majority of patients were from 60 to 79 age group (61 %, 63 % and 75 %, respectively). No sex-related difference between age groups was observed. More male patients (59 % of patients with nonadvanced adenomas, 66 % of patients with advanced adenomas, and 67 % of patients with early carcinomas) than female patients were enrolled in the study and the most frequent site of the adenomas was the rectum (46 %, 51 %, 59 %, respectively). No significant difference was observed in location between the two sexes. The size of adenomas was assessed by measuring the maximum diameter of the adenomatous component in millimeters on the histological slide and we compared this histological size to the size of the formalin-fixed macroscopic specimen. The result showed that, in 31 cases the size measured from histological slide, corresponded with the size of formalin-fixed specimen; in 79 cases the size of adenomas was smaller than the formalin-fixed specimen's size (smaller than 1–5 mm in 67 cases, smaller than 6–10 mm in 12 cases); in 19 cases the size was bigger than the formalin-fixed specimen's size (bigger than 1–5 mm in 17 cases, bigger than 6–10 mm in 2 cases). In 35 cases the size of the formalin-fixed specimen was greater than 25 mm and it cannot be represented on a single slide, therefore we used the measurement after fixation. These findings confirm that the measurement on histological slide is the most accurate. The well established interrelations between size, histological architecture and grade of dysplasia of colorectal adenomas [29] were reproduced by our data. The size of adenomas was significantly associated with the proportion of villous histology and grade of dysplasia (Table 3). The lesions ≥10 mm in size, were more likely to contain tubulovillous/villous feature**(s)** (106/113, 94 %) and a high grade dysplasia (82/113, 72 %). In contrast, the majority of minor (<10 mm) adenomas were tubular and more likely to show a low grade dysplasia (all $p<0.0001$).

### PCR-RFLP Assay Validation

The detection limit of mutation detection was determined by mixing wild-type DNA isolated from the K562 cell line with decreasing concentrations of mutated DNA were prepared from the homozygously mutated A549 cell line for codon 12, and from the heterozygous mutated HCT116 cell line for codon 13. The lowest level of detection was 3 % mutant DNA in the background of wild-type DNA as found in three independent experiments.

To establish the reproducibility of the mutation analysis [20], 30 colorectal adenoma specimens were subjected twice to complete procedure. In 100 % of the samples, the same KRAS status was observed in the duplicate experiments.

### KRAS Mutation Status

KRAS mutations were detected in 16/51 (31 %) of the non-advanced adenomas, in 65/113 (57.5 %) of the advanced

**Table 3** Pathological features of adenomas

| | | | Histology | | | Grade of neoplasia | | |
|---|---|---|---|---|---|---|---|---|
| Adenoma | Total | Tub | Tub/vill | Vill | $p$-value | LGMN | HGMN | $p$-value |
| Nonadvanced (<10 mm) | 51 | 28 | 19 | 4 | $p<0.0001$ | 31 | 20 | $p<0.0001$ |
| Advanced (≥10 mm) | 113 | 7 | 67 | 39 | | 31 | 82 | |
| Total | 164 | 35 | 86 | 43 | | 62 | 102 | |

adenomas and in 25/40 (62.5 %) of the early carcinomas. Most mutations presented at codon 12 rather than at codon 13 (72 %, 82 %, 76 % versus 22 %, 17 %, 24 % respectively). There was no significant difference in age, location within the colorectum among nonadvanced-, and advanced adenomas and early carcinomas. There was no association of KRAS

**Table 4** The correlation between KRAS mutation and clinicopathological features in colorectal adenoma and early carcinoma patients

| | *n* | Positive′ *n* (%) | Negative` *n* (%) | *p*-value° |
|---|---|---|---|---|
| Nonadvanced adenoma (<10 mm) | 51 | 16 (31) | 35 (69) | |
| Age (yr) | | | | |
| <60 | 14 | 4 (28.6) | 10 (71.4) | *ns* |
| ≥60 | 37 | 12 (32.4) | 25 (67.6) | |
| Gender | | | | |
| M | 31 | 7 (22.6) | 24 (77.4) | *ns* |
| F | 20 | 9 (45) | 11 (55) | |
| Location* | | | | |
| Proximal | 8 | 2 (25) | 6 (75) | *ns* |
| Distal | 19 | 5 (26) | 14 (74) | |
| Rectum | 23 | 8 (35) | 15 (65) | |
| Histology | | | | |
| Tub | 28 | 4 (14) | 24 (86) | $p=0.0065$ |
| Tub/vill | 19 | 11 (58) | 8 (42) | |
| Vill | 4 | 1 (25) | 3 (75) | |
| Grade of dysplasia | | | | |
| LGMN | 31 | 8 (26) | 23 (74) | *ns* |
| HGMN | 20 | 8 (40) | 12 (60) | |
| Advanced adenoma (≥10 mm) | 113 | 65 (57.5) | 48 (42.5) | |
| Age (yr) | | | | |
| <60 | 30 | 19 (63) | 11 (37) | *ns* |
| ≥60 | 83 | 46 (55) | 37 (45) | |
| Gender | | | | |
| M | 74 | 40 (54) | 34 (46) | *ns* |
| F | 39 | 25 (64) | 14 (36) | |
| Location** | | | | |
| Proximal | 15 | 12 (80) | 3 (20) | *ns* |
| Distal | 40 | 19 (47.5) | 21 (52.5) | |
| Rectum | 57 | 34 (60) | 23 (40) | |
| Histology | | | | |
| Tub | 7 | 2 (28.6) | 5 (71.4) | $p=0.0017$ |
| Tub/vill | 67 | 32 (48) | 35 (52) | |
| Vill | 39 | 31 (79) | 8 (21) | |
| Grade of dysplasia | | | | |
| LGMN | 31 | 22 (71) | 9 (29) | *ns* |
| HGMN | 82 | 43 (52) | 39 (48) | |
| Early carcinoma | 40 | 25 (62.5) | 15 (37.5) | |
| Age (yr) | | | | |
| <60 | 7 | 5 (71) | 2 (29) | *ns* |
| ≥60 | 33 | 20 (61) | 13 (39) | |
| Gender | | | | |
| M | 27 | 17 (63) | 10 (37) | *ns* |
| F | 13 | 8 (62) | 5 (38) | |
| Location*** | | | | |
| Proximal | 6 | 6 (100) | 0 | *ns* |
| Distal | 9 | 4 (44) | 5 (56) | |
| Rectum | 22 | 12 (55) | 10 (45) | |

′cases with Kras mutation; ″ cases with wild-type Kras; * location was not known for one Kras positive adenoma; ** location was not known for one Kras negative adenoma;*** location was not known for three Kras positive early carcinomas; ° comparing cases with Kras mutation to cases without Kras mutation;

*ns* – non significant

mutations with sex. The correlation between the activated KRAS oncogene and clinicopathological features in colorectal adenoma and early carcinoma patients is shown in Table 4.

KRAS mutation was found more often in tubulovillous and villous adenomas, whereas wild-type KRAS was observed more frequently in tubular adenomas ($P<0.0001$) and there was an increased prevalence of KRAS mutations in larger adenomas ($P<0.0001$) (Table 5).

Two adenomas contained both a codon 12 and a codon 13 mutation in their KRAS genes. The first case was a colon adenoma from 81-year old female patient, located in the cecum, 9 mm in size with tubulovillous histology and a high grade mucosal neoplasia. The second case was from 63-year old woman female patient, located in the sigmoid colon, 15 mm in size and with villous change, low grade neoplasia. Both adenomas revealed coexisting KRAS mutations - G12D (c.35 G-→A) and G13D (c.38 G-→A). Adenoma containing both codon 12 and 13 mutations in their KRAS gene is not a new finding and the meaning of double mutation in terms of pathogenesis of colorectal adenomas is not known.

## Discussion

Steps of transformation from normal epithelium to benign neoplasia (adenoma), followed by invasive carcinoma are described in the classic tumor progression model proposed by Fearon and Vogelstein [9]. Although most adenomas do not progress to cancer, even so every adenoma has the capacity for malignant transformation. The potential for malignant transformation is correlated to the size, histology and grade of dysplasia. Currently, based on these parameters, adenomas are characterized as "non-advanced and advanced" and patient surveillance is adjusted accordingly.

**Table 5** Relation between mutations of the KRAS gene and size, histology, grade of dysplasia of the adenoma

| Characteristics | No# with KRAS mutation | No# with KRAS wild-type | *p*-value |
|---|---|---|---|
| Size | | | |
| <10 mm | 16 | 35 | $p<0.0001$ |
| 10–19 mm | 32 | 37 | |
| ≥20 mm | 33 | 11 | |
| Histology | | | |
| Tub | 6 | 29 | $p<0.0001$ |
| Tub/vill | 43 | 43 | |
| Vill | 32 | 11 | |
| Grade of dysplasia | | | |
| LGMN | 30 | 32 | *ns* |
| HGMN | 51 | 51 | |

*ns*-non significant

The findings of our present study are similar to those of the literature; the most important morphological parameters of the adenoma such as size, histology, grade of dysplasia are highly correlated with one another: with increasing size there is a parallel increase in the proportion of villosity and degree of dysplasia.

The size of an adenoma is important for its risk of containing an adenocarcinoma and it is also related to the need for subsequent surveillance. An over or underestimation of adenoma size is important at the 10 mm threshold. *Schoen et al*. found that endoscopic estimates of adenoma size were inaccurate in 20 % of the cases compared to 3 % for pathologic measurements, with a tendency to overestimate the adenoma size [21]. An advantage of our study was that all adenomas were measured by pathologist on histological sections in mm as recommended by the European Guidelines [11]. We also found this technique simple to perform and more accurate.

KRAS mutations are associated with advanced adenomas [7, 8]. The frequency of KRAS gene mutations in colorectal adenomas reported in the literature, ranged from 20 % to 70 % [20, 23, 27, 33]. This broad range of reported frequencies may be due to various factors, such as geographical variations in the study populations in different countries, the use of different assay techniques, small series of selected patients or characteristics of examined adenomas. In this study, we identified KRAS mutations in 31 % of the non-advanced adenomas, in 57. 5 % of the advanced adenomas and in 62.5 % of the early carcinomas, developed within adenomas. These our findings are in general agreement with most studies (for instance, Burmer and Loeb reported a 75 % incidence in adenomas, McLellan et al. a 68 % incidence in adenomas and Vogelstein et al. a 50 % incidence in adenomas) and considerably higher than the incidences reported from Japan (Ando et al. reported 15 %) and the UK (Bell et al. reported 28 %) [27]. Also, according to the literature approximately 35 % of sporadic colorectal cancers have mutated KRAS [37]. In present study, the frequency of KRAS mutations was higher in advanced adenomas and pT1 cancers than in advanced CRC. The explanation for this paradoxical biological behavior is not fully understood. It have been suggested that mutated KRAS gene contributes to the transition of an intermediate adenoma to a late adenoma or carcinoma and KRAS mutation does not appear to be related to adenocarcinoma progression [7, 9, 20, 39].

In our study, there was no significant difference in association of KRAS mutation with age, gender, location between nonadvanced-, and advanced adenomas and early carcinomas. Furthermore, the correlation of KRAS mutation with adenoma size shows conflicting results in different studies. The fact that the KRAS mutation occurs at an intermediate stage of the adenoma progression to carcinoma was established by Vogelstein et al. [24]. Studies since then

have confirmed that the frequency of KRAS mutation in adenomas relates significantly to larger adenoma size [7, 25, 34, 35]. This is in contrast to the reports of other studies that found that KRAS mutations were not correlated with the size of the adenoma [23, 27, 28, 36]. In our study, we found that the incidence of activated KRAS oncogene is significantly associated with the size of colorectal adenomas ($P<0.0001$) (Table 5). Ras mutations are thought to be an earlier event in sporadic adenoma formation and the used technique for the adenoma size assessment may influence the conflicting results.

KRAS mutation is very strongly associated with villous architecture and similar to those being reported previously [7, 23, 25, 28, 34, 36]. We found an increased prevalence of KRAS mutations in villous or tubulovillous adenomas compared with tubular adenomas (74 %, 50 % versus 17 % respectively, $p<0.0001$). Moreover, *Jass et al*. have reported that the KRAS mutation was significantly more frequent in adenomas that included a villous architecture (50 %) and *Ishii et al.* have demonstrated a highly significant relationship between villosity and KRAS mutation. They have identified KRAS mutation in 17.9 % (12/69) of tubular adenomas without villous change, in 59 % (46/78) of tubular adenomas with villous component up to 20 % and in 78.4 % (91/116) of tubulovillous adenomas [28, 37]. We would agree with the suggestion that KRAS mutation is linked with the development of villous change. Also, KRAS mutation (especially G →A transitions) confer to sporadic adenomas an increased risk of tumor progression through villous component expansion [36]. In adenomas with villous configuration, the risk of malignant progression is much higher than in tubular adenomas [26, 29] and villous adenomas have a trend for an increased risk of recurrent advanced adenomas [5, 38]. The grade of dysplasia is highly predictive of the malignant transformation of adenomas [29, 30]. However, the findings of our study are similar to those of Risio et al. [36], namely the grade of dysplasia was independent from the KRAS mutation and from the villous structure. In this study KRAS mutation occurred with the same frequency in adenomas with low-grade (48 %) and high-grade (50 %) dysplasia.

Patients with advanced adenomas are at a high risk for recurrent advanced adenomas and should have surveillance colonoscopy at a 3-year interval. In the study by Tsai et al. [22] the majority (69 %) of advanced adenomas are <10 mm. Even among polyps≤5 mm, there was an appreciable prevalence of advanced adenomas (10 %). In our study, among the nonadvanced adenomas **by size**, there were 20/51 (39 %) adenomas with a high grade dysplasia and 23/51 (45 %) adenomas with tubulovillous-villous structure.

In conclusion, KRAS mutation is very strongly associated with a villous architecture and through villous component expansion, KRAS mutations may increase risk of tumor progression in sporadic colorectal polypoid adenomas. As the potential for adenoma progression with a single molecular marker is difficult to assess, further studies should be conducted to identify new biomarkers that may help to identify the increased risk of neoplastic progression and will help to improve surveillance.

**Conflicts of Interest** The authors disclose no conflicts.

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