p53 Up-regulation During Colorectal Carcinogenesis

CARLOS A. RUBIO, MARGARETA RODESJÖ, ANNIKA DUVANDER, MARIANNE MATHIES, LISA GARBERG and JAYANT SHETYE

Department of Pathology, Karolinska Institute and University Hospital, Stockholm, Sweden

Abstract. Background: We previously found foci of p53 up-regulation in dysplasia in colorectal adenomas (CRAs). The present study aimed at exploring the frequency of this phenomenon in CRAs with and without submucosal invasive carcinoma. Materials and Methods: Sections from 568 polypectomies or surgical resections harbouring a CRA (without or with submucosal invasion) or overt colorectal carcinomas were challenged with p53 immunostaining. The largest section from single colorectal neoplasias was measured by the aid of a calibrated ocular scale in a conventional microscope. Lesions were divided into small adenomas (≤10 mm in size), large adenomas (≥11 mm in size), adenomas with submucosal invasion, and overt invasive carcinomas (without any recognizable adenoma remnant tissue). Results: CRAs with three or more dysplastic foci of p53-up-regulation gradually increased from 8% in small adenomas (size: ≤10 mm) to 48% in large adenomas (size: ≥11 mm), and to 65% in the adenomatous tissue in adenomas displaying submucosal invasion), but plummeted to 13% in the submucosal carcinomatous tissue and to 11% in overt carcinomas. In contrast, extensive p53 up-regulation predominated in the submucosal carcinomatous tissue (87%) and in overt carcinomas (89%). Conclusion: The frequency of foci of dysplastic glands with up-regulation of p53 (hotspots) gradually increased from small to larger CRAs, being highest in the adenomatous tissue of CRAs with submucosal invasive carcinoma. The foci of p53 up-regulation became confluent (appreciated as extensive up-regulation) in the submucosal carcinomatous tissue and in overt carcinomas. It is concluded that a high number of foci with p53 up-regulation in adenomatous tissue might be required before submucosal invasive carcinoma ensues.

Correspondence to: C.A. Rubio, MD, Ph.D., Gastrointestinal and Liver Pathology Research Laboratory, Department of Pathology, Karolinska Institute and University Hospital, 17176, Stockholm, Sweden. Fax: +46 851774524, e-mail: Carlos.Rubio@ki.se

Key Words: Colorectal adenomas, p53, focal up-regulation, invasive carcinoma.

Colorectal adenomas (CRAs) are foci of highly proliferating dysplastic cells with biochemical and molecular alterations carrying a particular propensity to progress to colorectal carcinoma (CRC) (1). The colorectal adenoma–carcinoma sequence embodies a well-known paradigm for sequential cancer development driven by the accumulation of genomic mutations. Years ago Vogelstein *et al*. proposed a multi-step molecular paradigm of colorectal tumorogenesis, whereby, following Adenomatous polyposis coli (APC) (5q21) inactivation, accumulation of β-catenin in the nucleus, hypomethylation and V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations, tubular adenomas with low-grade dysplasia progressed to villous adenomas with high-grade dysplasia (2). Allelic loss of the chromosome 17p region containing the *p53* gene in conjunction with mutation of the second allele would give rise to bi-allelic inactivation. This functional loss of p53 in CRC was proposed to be a late event in the transition from adenoma to carcinoma.

The p53 transcription factor (encoded by the human gene *TP53*) is a key tumor suppressor, as it regulates several signaling pathways involved in carcinogenesis (3). The wild-type *TP53* gene hosts the vital anti-proliferative function that empowers cells to arrest their proliferation cycle in G_1, aiming to repair the DNA of highly damaged cells and to prevent the expansion of potential cancer clones. Cells that fail to repair their DNA undergo apoptosis, but when the wild-type *p53* is inactivated by the mutant *p53*, this mechanism collapses (4).

Mutations in *TP53* have been found in 50% of patients with colonic cancer (5). Given the logistical difficulties and resources associated with direct sequencing of the *TP53* gene, most investigations have used immunohistochemistry as a means of detecting mutant p53, with the assumption that overexpression of p53 is often associated with a mutation, while the lack of expression is generally indicative of wild-type p53 (6).

According to the prevailing theory, most CRCs arise from adenomas. Nonetheless, histological studies have demonstrated that the majority of the CRAs are indolent neoplasias in which invasive carcinoma does not occur. In fact, only 1% to 3% out

of endoscopically-resected CRAs of all sizes revealed invasive carcinoma at histological examination (7). In contrast, the study of 926 CRAs demonstrated in several surveys (8-17) that p53-up-regulation occurs in up to 64% of the cases (8). Hence, despite extensive studies of the *TP53* gene and of its encoded protein p53 in colorectal carcinogenesis, our understanding of why only occasional indolent CRAs are selected to progress to invasive carcinoma remains elusive.

In a preliminary study, we found haphazardly distributed foci of p53 up-regulation in CRAs (18). It was speculated that these foci could highlight autonomous groups of dysplastic glands supposedly committed to cancer progression.

The purpose of the present work was to explore whether the frequency of foci of p53 up-regulation correlated with the presence of submucosal invasive carcinoma in a large cohort of CRAs.

Materials and Methods

The study comprised of 568 non-consecutive polypectomies or surgical resections harbouring a CRA with or without submucosal invasion, as well as overt CRCs.

The Institutional Review Board approved this retrospective study. No individual patient identification was involved and no study-driven clinical intervention was performed.

CRAs with invasive carcinoma were regarded as those exhibiting neoplastic cells or glands invading through the *muscularis mucosae* into the submucosal tissues, without reaching the confines of the margin of resection. In CRAs with invasive carcinoma, the term adenomatous tissue is used to define the adenomatous area; the area with invasive carcinoma was separately analysed, appearing as such in results and Tables.

Overt carcinomas were regarded those invasive carcinomas without any recognizable adenoma remnant tissue (19).

Sections were challenged with the primary mouse monoclonal antibody (IgG1, kappa) directed against human p53 protein (anti-p53, DO-7; Ventana Medical System, Inc, Roche, Switzerland).

Histological evaluation. CRAs were classified according to their structural configuration into tubular adenomas (TA), villous adenomas (VA), and traditional serrated adenomas (TSA), and according to their degree of cellular dysplasia into: low-grade dysplasia (LGD), high-grade dysplasia (HGD) (20-22).

Size assessment. Previous investigations revealed that polyp size assessment by endoscopists, radiologists and pathologists was unreliable when compared to the gold standard size (23-25). To by-pass this problem, the largest section from single colorectal neoplasias was measured by the aid of a calibrated ocular scale in a conventional microscope. Lesions were divided into small adenomas (≤10 mm in size), large adenomas (≥11 mm in size), adenomas with submucosal invasion, and overt invasive carcinomas (without any recognizable adenoma remnant tissue).

To illustrate this work, the largest section exhibiting p53-up-regulation was scanned in an EPSON Perfection 4990 (Epson America, Inc., Long Beach, CA, USA), together with a sidewise placed conventional ruler. Hence, the illustrations presented in Figures 1-7, provide the reader with the actual size of the lesion scanned.

Scoring p53 expression. Dysplastic cells or tumour cells were regarded as overexpressing the p53 protein when strongly immunostained.

p53-up-regulation was found either focally or extensively distributed. Focal p53 was present as a single assemblage or focus of dysplastic glands, or as several foci (assemblages) of dysplastic glands. Extensive p53 up-regulation was noted when present in the entire adenomatous tissue, in the entire submucosal invasive carcinoma, or in the entire cancerous tissue in overt carcinomas.

Statistical analysis. Kruskal–Wallis one-way analysis of variance was used to compare difference between groups. Statistical significance was defined as $p<0.05$.

Results

Size of CRAs. Out of the 568 neoplastic lesions investigated, 38% (n=215) were small adenomas (mean= 8 mm, range=4-10 mm), 38% (n=218) large adenomas (mean= 18 mm, range=11-28 mm), 12% (n=68) were adenomas displaying submucosal invasion (mean=16 mm, range=8-30 mm), the remaining 12% (n=67), being overt carcinomas. Out of the 68 adenomas with submucosal invasion, 88.2% (n=60) measured ≥11 mm (mean=17 mm, range=12-27 mm).

Histological phenotype of CRAs. Out of the 568 neoplastic lesions reported in Table I, 88% (n=501) were CRAs. Histology revealed that the majority of small adenomas (58%) were TA with LGD, whereas the majority of large adenomas (51%) and adenomas with submucosal invasion (57%) were VA with HGD.

Frequency of p53 up-regulation in different colorectal neoplastic lesions. From Table II, it may be deduced that the p53 protein was up-regulated in 28% (141/501) of the adenomas/adenomatous tissue, and in 51% (69/135) of the invasive carcinomas, either in the submucosa or in overt carcinomas; (p53 up-regulation in CRAs *vs*. carcinomas, $p<0.05$).

In small adenomas, *p53* up-regulation was recorded in 6%, in 45% of large adenomas, and in 46% in the adenomatous tissue (Table II) (p53 up-regulation small adenomas *vs*. large adenomas/adenomatous tissue, $p<0.05$). p53 was found up-regulated in 34% of the submucosal invasive carcinomas, and in 69% of the overt carcinomas ($p<0.05$).

Distribution of p53 up-regulation in colorectal neoplastic lesions. i) Focal p53 up-regulation (Figures 1-5): Out of the 210 lesions exhibiting p53 up-regulation reported in Table III, focal up-regulation was found in 57% (n=119): in 83% of small adenomas, in 80% of large adenomas, and in 74% in the adenomatous tissue, but only in 13% in submucosal

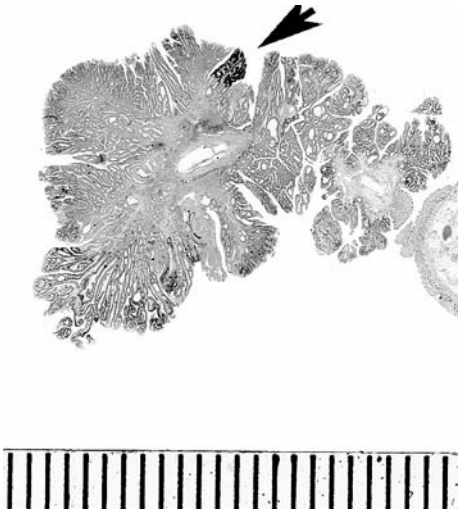

Figure 1. *Colonic adenoma with one focus of p53 up-regulation (arrow). Endoscopic polypectomy, p53 immunostain. Section was scanned with EPSON Perfection 4990; scale in mm.*

Figure 3. *Colonic adenoma with three foci of p53 up-regulation (arrows). Endoscopic polypectomy, p53 immunostain. Section was scanned with EPSON Perfection 4990; scale in mm.*

Figure 2. *Rectal adenoma with two foci of p53 up-regulation (arrows). Endoscopic polypectomy, p53 immunostain. Section was scanned with EPSON Perfection 4990; scale in mm.*

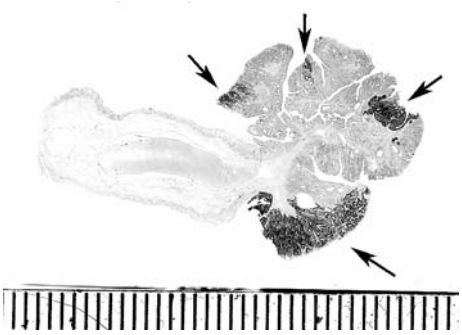

Figure 4. *Colonic adenoma with four foci of p53 up-regulation (arrows). Endoscopic polypectomy. p53 immunostain. Section was scanned with EPSON Perfection 4990; scale in mm.*

carcinomas and 11% in overt carcinomas (focal p53-up-regulation in adenomas/adenomatous tissue *vs*. submucosal carcinoma/overt carcinomas, $p \leq 0.05$).

Multiple foci with p53 up-regulation: From Table IV it may be deduced that ≥3 up-regulated p53 foci were found in 8% (1/12) of the small adenomas, in 48% (47/98) of the large adenomas, in 65% (20/31) of the adenomatous tissue, but only in 13% (3/23) of the submucosal-invasive carcinomas and in 11% (5/46) of the overt carcinomas, (≥3 p53 up-regulated foci in large adenomas/ adenomatous tissue *vs*. small adenomas/ submucosal invasion/ overt carcinomas, $p \leq 0.05$).

ii) Extensive p53 up-regulation (Figures 6 and 7). Out of the 210 lesions exhibiting p53 up-regulation in Table V, extensive p53 up-regulation was found in 43% (n=91): in 21% (30/141) of small and large adenomas/adenomatous tissue, and in 88% (59/67) of submucosal invasion/overt carcinomas (p53 up-regulation in CRAs *vs*. invasive carcinomas, $p<0.05$). This difference was retained when the various groups were separately analyzed in Table VI; extensive p53 up-regulation was recorded in 17% of small adenomas, in 20% of large adenomas, in 26% of the adenomatous tissue, but in 86% in submucosal invasion and in 89% in overt carcinomas ($p<0.05$).

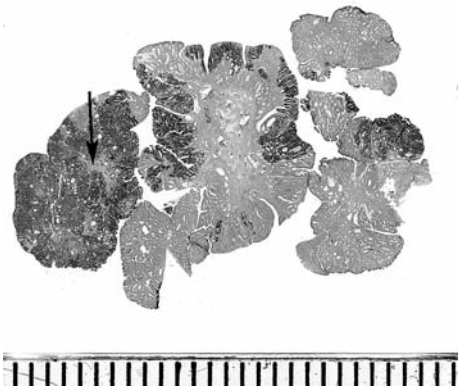

Figure 5. *Colonic adenoma with multiple foci of p53 up-regulation with submucosal carcinoma (arrow). Endoscopic polypectomy. p53 immunostain. Section was scanned with EPSON Perfection 4990; scale in mm.*

Figure 6. *Colonic adenoma with confluent foci of p53 up-regulation with carcinoma invading into the muscularis propria (arrow). Surgical specimen. p53 immunostain. Section was scanned with EPSON Perfection 4990; scale in mm.*

Figure 7. *Overt carcinoma of the colon with extensive p53-up-regulation. Surgical specimen. p53 immunostain. Section was scanned with EPSON Perfection 4990; scale in mm.*

Table I. *Histology in 568 colorectal neoplastic lesions: 501 colorectal adenomas (CRAs) and 67 overt invasive carcinomas.*

Histology	Small adenomas	Large adenomas	Adenomas with submucosal invasion
TALGD	124 (57.7%)	19 (8.7%)	
TAHGD	29 (13.5%)	46 (21.1%)	22 (32.4%)
VALGD	26 (12.1%)	17 (7.8%)	
VAHGD	21 (9.8%)	110 (50.5%)	39 (57.4%)
TSALGD	10 (4.7%)	14 (6.5%)	
TSAHGD	5 (2.3%)	12 (5.5%)	7 (10.3%)
Total	215 (100%)	218 (100%)	68 (100%)

TALGD: Tubular adenoma with low-grade dysplasia, TAHGD: tubular adenoma with high-grade dysplasia, VALGD: villous adenoma with low-grade dysplasia, VAHGD: villous adenoma with high-grade dysplasia, TSALGD: traditional serrated adenomas with low-grade dysplasia, TSAHGD: traditional serrated adenomas with high-grade dysplasia.

Table II. *Frequency of p53 up-regulation in 568 colorectal neoplastic lesions.*

Histologic group	p53 up-regulation/total cases (%)
Small adenomas	12/215 (5.6%)
Large adenomas	98/218 (44.9%)
Adenomas with submucosal invasion	
Adenomatous tissue	31/68 (45.6%)
Submucosal invasive carcinoma	23/68 (33.8%)
Overt carcinomas	46/67 (68.6%)
Total	210/568 (36.9%)

Table III. *Focal p53 up-regulation in 210 colorectal neoplastic lesions exhibiting p53 up-regulation.*

Histologic groups	Focal p53 up-regulation/ Total cases (%)
Small adenomas	10/12 (83.3%)
Large adenomas	78/98 (79.6%)
Adenomas with submucosal invasion	
adenomatous tissue	23/31 (74.2%)
submucosal invasion	3/23 (13.0%)
Overt carcinomas	5/46 (11.1%)
Total	119/210 (56.7%)

Discussion

The present investigation showed that the frequency of dysplastic glands with foci of p53 up-regulation significantly increased from 6% in small adenomas, to 36% in large adenomas and to 69% in the adenomatous tissue. *Pari passu*, the frequency of neoplastic lesions with three or more

Table IV. *The number of foci with p53 up-regulation in 210 colorectal neoplastic lesions exhibiting p53 up-regulation.*

Histological groups	No. of foci with p53 up-regulation				No. cases
	1	2	3	≥4	
Small adenomas	8	1	1	0	12
Large adenomas	8	23	26	21	98
Adenomas with submucosal invasion					
Adenomatous tissue	1	2	4	16	31
Submucosal invasion	0	0	1	2	23
Overt carcinomas	0	0	0	5	46
Total	17	26	32	44	210

Table V. *Extensive p53 up-regulation in 210 colorectal neoplastic lesions exhibiting p53 up-regulation*

Histological groups	Extensive p53 up-regulation/ Total cases (%)
Small adenomas	2/12 (16.7%)
Large adenomas	20/98 (20.4%)
Adenomas with submucosal invasion	
adenomatous tissue	8/31 (25.8%)
submucosal invasion	18/21 (85.7%)
Overt carcinomas	41/46 (89.1%)
Total	89/210 (42.3%)

Table VI. *Publications on p53 up-regulation in 926 colorectal neoplastic lesions.*

Authors (ref)	p53-Positive CRAs and carcinomas
Sheikh *et al*. (8)	64% of 42 CRAs
Sundblad *et al*. (9)	45% of 58 CRAs
Nogueira *et al*. (10)	60% of 72 CRAs
Saleh *et al*. (11)	37% of 49 CRAs; 65% of 20 carcinomas
Yao *et al*. (12)	12% of 50 VA; 18% in invasive components
Mueller *et al*. (13)	42% of 50 CRAs
Visca *et al*. (14)	17% of 100 CRAs; 44% of 100 CRCs
Watatani *et al*. (15)	2% in 333 CRAs
Speroni, *et al*. (16)	53% of 100 CRAs
Kaklamanis *et al*. (17)	26% of 72 CRAs

CRA: Colorectal adenoma; VA: villous adenoma.

dysplastic foci of p53 up-regulation (hotspot mutations) gradually increased from 8% in small adenomas, to 48% in large adenomas, and to 65% in the adenomatous tissue. Thus, the highest proportion of dysplastic cells with p53 up-regulation was recorded in the adenomatous tissue synchronously exhibiting submucosal invasion. In light of these findings, we are prone to speculate that p53 up-regulation might had reached " the point of no-return" in the adenomatous tissue, an event that might have sent signals aimed to trigger tumour penetration into the submucosal tissues. In contrast, extensive p53 up-regulation predominated in tissues with invasion: in 87% with submucosal invasion and in 89% of overt carcinomas, and focal p53 up-regulation plummeted to 13% and to 11%, respectively. The rational explanation for this phenomenon might be that the foci of p53 up-regulation became confluent (extensive) in invasive carcinomas.

Several authors studied the frequency of p53 up-regulation in CRAs (cfr. Table VI). The Table shows that that the percentage of CRAs with p53 up-regulation varied from 2% in initial adenomas (15) to 64% (8). For some authors, p53 staining was recorded as positive irrespective of the percentage of positive glands (15), whereas for others, p53 staining above 10% was considered positive (9). It is apparent that the various criteria used to define p53 up-regulation in CRAs might have influenced the disparate results in the literature.

According to Rivlin *et al*., mutations of the *TP53* gene occurring at the early stages of tumourigenesis fuel uncontrolled proliferation, whereas *TP53* mutations at later stages, synergizing with additional oncogenic events, trigger invasion (3). Our findings suggest that accumulation of p53 up-regulated foci of dysplastic cells in CRAs should reach a substantial level before invasion ensues.

Puzzlingly, two cases having three or more foci of p53 up-regulation in the adenomatous tissue showed absence of p53 immunoreactivity in the submucosal invasive tissue. Although the cause of this conundrum remains unclear, the possibility that p53 protein expression might have been down-regulated in the invasive tissue by one or more molecular signals cannot be totally rejected. One possible candidate for this down-regulation might be the double minute 2 homolog (MDM2) also known as E3 ubiquitin-

protein ligase Mdm2 that plays a key role in regulating p53 protein (26). MDM2 binds to p53 within the p53 transactivation domain, interfering with the enrolment of transcription components. MDM2 binding can actively repress transcription when bound to p53 and through proteolytic degradation may completely eliminate p53 (27, 28). Another candidate is the Transcriptional repressor CTCF also known as 11-zinc finger protein or CCCTC-binding factor, a zinc finger protein that hampers the *TP53* promoter from accumulating repressive histone marks (29). When the *TP53* promoter stores such histone marks, however, the CTCF protein binds the p53, resulting in the decrease of p53 expression (29). More recently, Suzuki H *et al*. found in CRCs that the epigenetic inactivation by methylation of insulin-like growth factor-binding protein 7 (IGFBP7) correlated with the serine/threonine-protein kinase B-Raf mutations, the presence of the CpG island methylator phenotype (CIMP) and the absence of *TP53* mutations (4). Accordingly, one or more of these candidate signals might account for the absence of p53 up-regulation in the invasion tissue in the two CRAs exhibiting p53 up-regulation in the adenomatous tissue.

In the present survey, some CRAs exhibiting foci of p53 up-regulation in dysplastic glands with HGD occasionally exhibited p53 up-regulation in glands with LGD. The latter finding suggests that p53 up-regulation might not be exclusively linked to the most severe degree of dysplastic aberration, but possibly to particular putative cellular receptor(s) present in autonomous groups of dysplastic cells.

In sum, foci of p53 up-regulation (hot spots) in dysplastic glands gradually increased, from small to large adenomas, being highest in the adenomatous tissue in adenomas with submucosal invasive carcinoma. In carcinomas (submucosal and overt), the foci of p53 up-regulation became confluent (appreciated as extensive p53-up-regulation).

It is submitted that a high number of foci with p53 up-regulation might be necessary in CRAs before submucosal invasive carcinoma ensues. Quantification of the number of such hotspots might be a useful biological marker for assessing cancer risk in CRA.

The review of the literature indicates that this is the first study showing that multiplicity of foci with p53 up-regulation in CRAs might be required before submucosal invasive carcinoma develops.

Conflicts of Interest

The Authors state that they have no conflict of interest.

Acknowledgements

The Authors would like to thank Dr. Lorand Kis for valuable comments and constructive criticisms of the manuscript.

References

1 Jackman C and Mayo CW: The adenoma–carcinoma sequence in cancer of the colon. Surg Gynecol Obstet *93*: 327-330, 1951.
2 Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM and Bos JL: Genetic alterations during colorectal-tumor development. N Engl J Med *319*: 525-528, 1988.
3 Rivlin N, Brosh R, Oren M and Rotter V: Mutations in the p53 tumor-suppressor gene: important milestones at the various steps of tumorigenesis. Genes Cancer *2*: 466-474, 2011.
4 Suzuki H, Igarashi S, Nojima M, Maruyama R, Yamamoto E, Kai M, Akashi H, Watanabe Y, Yamamoto H, Sasaki Y, Itoh F, Imai K, Sugai T, Shen L, Issa JP, Shinomura Y, Tokino T and Toyota M: IGFBP7 is a p53-responsive gene specifically silenced in colorectal cancer with CpG island methylator phenotype. Carcinogenesis *31*: 342-349, 2010.
5 Saleh HA, Jackson H and Banerjee M: Adenomas of the colon: overexpression of p53 protein and risk factors. Endoscopy *30*: 623-626, 1998.
6 Theodoropulous GT, Karafoka E and Papailiu J: P53 and EGFR expression in colorectal cancer: A reappraisal of 'old' tissue markers in patients with long follow-up. Anticancer Res *29*: 785-791, 2009.
7 Strock P, Mossong J, Scheiden R, Weber J, Heieck F and Kerschen A: Colorectal cancer incidence is low in patients following a colonoscopy. Dig Liv Dis *43*: 899-904, 2011.
8 Sheikh RA, Min BH, Yasmeen S, Teplitz R, Tesluk H, Ruebner BH, Tobi M, Hatfield J, Fligiel S and Lawson MJ: Correlation of Ki-67, p53, and Adsab-9 immunohistochemical staining and ploidy with clinical and histopathologic features of severely dysplastic colorectal adenomas. Dig Dis Sci *48*: 223-229, 2003.
9 Sundblad AS, Chumbita RL and Zoppi JA: Expression of p53 and Bcl-2 in colorectal adenomas and carcinomas. Medicina *57*: 662-666, 1997.
10 Nogueira RB, Pires AR, Soares TM, Rodrigues SR, Rodrigues SR, Campos MA, Toloi GC and Waisberg J: Immunoexpression of the COX2, p53, and caspase-3 proteins in colorectal adenoma and non-neoplastic mucosa. Einstein *11*: 456-461, 2013.
11 Saleh HA, Aburashed A, Bober P and Tabaczka P: P53 protein immunohistochemical expression in colonic adenomas with and without associated carcinoma. Am J Gastroent *93*: 980-984, 1998.
12 Yao T, Kajiwara M, Kouzuki T, Iwashita A and Tsuneyoshi M: Villous tumor of the colon and rectum with special reference to roles of p53 and Bcl-2 in adenoma–carcinoma sequence. Appl Immunohistochem Mol Morphol *8*: 175-182, 2000.
13 Mueller J, Mueller E, Hoepner I, Jütting J, Bethke B, Stolte M and Höfler H: Expression of Bcl-2 and p53 in *de novo* and ex-adenoma colon carcinoma: a comparative immunohistochemical study. J Pathol *180*: 259-265, 1996.
14 Visca P, Alò PL, Del Nonno F, Trombetta G, Marandino F, Filippi S, Di Tondo U and Donnorso RP: Immunohistochemical expression of fatty acid synthase, apoptotic-regulating genes, proliferating factors, and ras protein product in colorectal adenomas, carcinomas, and adjacent non-neoplastic mucosa. Clin Cancer Res *5*: 4111-4118, 1999.
15 Watatani M, Ieda S, Kuroda K, Inui H, Nishimura K and Yasutomi M: Comparison of p53 and Bcl-2 expression in initial, synchronous, and metachronous colorectal adenomas. Surg. Today *29*: 707-712, 1999.

16 Speroni AH, Vanzulli SI and Meiss RP: Adenomas of the colon: overexpression of p53 protein and risk factors. Endoscopy *30*: 623-626, 1998.
17 Kaklamanis L, Gatter KC, Mortensen N, Baigrie RJ, Heryet A, Lane DP and Harris AL: p53 expression in colorectal adenomas. Am J Pathol *142*: 87-93, 1993.
18 Rubio CA: Luminal histological outline and colonic adenoma phenotypes. Anticancer Res *27*: 3555-3559, 2007.
19 Rubio CA: Further studies on the arrest of cell proliferation in tumor cells at the invading front of colonic adenocarcinoma. J Gastroenterol Hepatol *22*: 1877-1881, 2007.
20 Rubio CA, Kristjansdottir S, Thodleifsson B, Olafsdóttir E, and Jonasson JG: The frequency of advanced adenoma in consulting patients: a nationwide survey in Iceland (2003-2006). Colorectal Dis *14*: e595-602, 2012.
21 Rubio CA and Rodensjö M: p53 overexpression in flat serrated adenomas and flat tubular adenomas of the colorectal mucosa. J Cancer Res Clin Oncol *121*: 571-576, 1995.
22 Rubio CA and Jaramillo E: Advanced microtubular colorectal adenomas: a 10-year survey at a single hospital. Anticancer Res *33*: 5471-5476, 2013.
23 Rubio CA, Höög CM, Broström O, Gustavsson J, Karlsson M, Moritz P, Stig R, Wikman O, Mattsson L and Palli D: Assessing the size of polyp phantoms in tandem colonoscopies. Anticancer Res *29*: 1539-1545, 2009.
24 Suzuki C, Matsson L and Rubio CA: Assessing polyp size by improved digitalized computed tomography (CT). Anticancer Res *28*: 1911-1915, 2008.
25 Rubio CA, Jónasson JG, Nesi G, Mazur J and Olafsdóttir E: The size of colon polyps revisited: intra- and inter-observer variations. Anticancer Res *30*: 2419-2423, 2010.
26 Ray RM, Bhattacharya S and Johnson LR: MDM2 inhibition induces apoptosis in p53 deficient human colon cancer cells by activating p73- and E2F1-mediated expression of PUMA and SIVA-1. Apoptosis *16*: 35-44, 2011.
27 Meng RD, Shih H, Prabhu NS, George DL and el-Deiry WS: Bypass of abnormal MDM2 inhibition of p53-dependent growth suppression. Clin Cancer Res *4*: 251-259, 1998.
28 Okayama S, Kopelovich L, Balmus G, Weiss RS, Herbert BS, Dannenberg AJ and Subbaramaiah K: p53 protein regulates Hsp90 ATPase activity and thereby Wnt signaling by modulating Aha1 expression. J Biol Chem *289*: 6513-6525, 2014.
29 Saldaña-Meyer R, González-Buendía E, Guerrero G, Narendra V, Bonasio R, Recillas-Targa F and Reinberg D: CTCF regulates the human p53 gene through direct interaction with its natural antisense transcript, WRAP53. Genes Dev *28*: 723-734, 2014.

Received July 31, 2014
Revised September 9, 2014
Accepted September 15, 2014