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Tissue distribution of lipid peroxidation product acrolein in human colon carcinogenesis

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Abstract

Lipid peroxidation product acrolein, well-known pollutant in tobacco and automotive smoke, accumulates *in vivo* bound to proteins. It suppresses p53 synthesis acting as potent carcinogenic factor for oral, respiratory and bladder carcinomas, while its possible association with colon carcinogenesis was not studied so far. We used genuine monoclonal antibody to evaluate immunohistochemical distribution of acrolein–protein adducts in 113 human colon tumours. The presence of acrolein–protein adducts was increasing with respect to colon carcinogenesis, from moderate appearance in tubular and villotubular low-grade adenomas to abundant and diffuse distribution in high-grade villotubular adenomas and Dukes A carcinomas. However, in advanced Dukes B and C carcinomas acrolein was hardly noticed, although, its protein adducts were found abundant in non-malignant colon epithelium of these patients. There was no relationship between p53 and acrolein distribution. According to these findings, acrolein seems to be lipid peroxidation product associated with transition from benign into malignant colon tumours.

Keywords: *Lipid peroxidation, acrolein, p53, 4-hydroxynonenal, oxidative stress, colon carcinogenesis*

Introduction

Cancers of the colon and rectum are rare in developing countries, but are the second most frequent malignancy in affluent societies. More than 940.000 cases occur annually worldwide, and nearly 500.000 die from it each year [1]. Both, diet and genetic factors, play key roles in its aetiology. While genetic predisposition is only sometimes well defined, like in case of familiar adenomatous polyposis, dietary factors are considered to have major (co)carcinogenic impacts [2].

Epithelial polyps that arise as the result of proliferation and dysplasia are termed adenomatous polyps or adenomas. They are true neoplastic lesions and posses a malignant potential as precursor lesions to colon adenocarcinoma. Histopathologically there are two types of neoplastic polyps: tubular adenomas and villous adenomas. These two types of tumours may be intermixed, resulting in mixed tubulovillous adenomas. Benign tubular adenomas have small but certain risk of malignant transformation. Villous adenomas have a high propensity for malignant transformation, which is recognised in early stages by the irregularity of glands that invade the muscularis. The risk of harbouring *in situ* or invasive carcinoma generally correlates with the proportion of the lesion that is villous, while the development of colon carcinoma from adenomatous lesions is referred

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to as the adenoma–carcinoma sequence [2]. Animal models also demonstrate that high dietary fat increases the number and the size of pre-malignant adenomas as well as number of spontaneous colon carcinomas [3,4].

Neoplastic transformation is a long-term process from normal to malignant cell, during which many intracellular molecular mechanisms occur altering structure and function of specific tumour suppressor genes or proto-oncogenes [5–9]. However, only a minor proportion of genetic lesions found in tumours are due to inheritance. Environmental factors are much more likely to cause the alteration [10]. The permanent oxidative damage, overproduction of reactive oxygen species (ROS) and consequently macromolecular damage often result in initiation and/or promotion of tumour tissue [11–13]. In pathology of oxidative stress, crucial process is often lipid peroxidation, which depends a lot on the dietary factors. A case control study of colorectal cancer in a multiethnic population suggests that the ratio of polyunsaturated to saturated fat may be a better indicator of colorectal cancer risk than the absolute amount of specific fats in the diet [14]. Persistent oxidative stress in colorectal carcinoma patients with decreased concentration of antioxidant vitamins together with lower amount of uric acid may be responsible for the formation of pro-oxidative environment in these patients [15].

Kondo et al. proposed that colorectal carcinoma, but not adenoma cells, are exposed to more oxidative stress than corresponding non-tumorous epithelial cells regardless of clinical stage and histology and that oxidative stress in carcinoma cells might stimulate cellular proliferation [16]. This may be due to lipid peroxidation product 4-hydroxy-2-nonenal (HNE) considered as "second toxic messenger of free radicals" [17]. Namely, in high concentrations, HNE is cytotoxic and mutagenic, while in physiological concentrations this aldehyde acts as a growth-modifying factor that interferes with cytokines (such as TGFβ1, EGF and PDGF) influencing activities of proto-oncogenes and regulating proliferation, differentiation and apoptosis [18–20].

Reactions of ROS with polyunsaturated fatty acids of membrane lipids do not result in production of only HNE but also a variety of reactive aldehydes, such as malondialdehyde and acrolein that react with various bioactive macromolecules including proteins and nucleic acids and alter their structure and function. Acrolein is aldehyde that is well known as an ubiquitous pollutant in environment being a product of incomplete combustion of plastic material, cigarette smoking and overheating frying oils. Moreover, this aldehyde is a metabolite of allyl compounds biotransformation and also of the widely used anticancer drug cyclofosphamide [21]. Among all α,β-unsaturated aldehydes including HNE and malondialdehyde, acrolein is the strongest electrophile and therefore shows the highest reactivity toward proteins and DNA [22].

Acrolein modulates harmful effects of Benz(a) pyrene, major carcinogen found in smoke and plays important role in initiation of lung cancer inhibiting the tumour suppressor activity of p53 [23]. The ability of acrolein to transcriptionaly activate genes responsible for phase II enzymes may form the basis of resistance against cell death and can have implications in cigarette smoke related lung carcinogenesis [24]. Thus, acrolein may be considered as a potent (co)carcinogen, however, its possible association with colon carcinogenesis was not evaluated until now.

In this article, we give evidence that acrolein is differentially distributed in colon adenomas and in colorectal carcinomas in dependence of clinical stage and histological grading of these tumours.

Materials and methods

Tumours

We analysed 24 tubular adenomas, 24 low-grade villotubular adenomas, 20 high-grade villotubular adenomas and 45 colorectal carcinomas. Nineteen of them were graded as Dukes A, 11 Dukes B and 15 Dukes C. These grades correspond to grade T1, T2, T3 and T4 according to the World Health Organisation classification [2]. Colorectal cancers are usually staged according to the Dukes classification or its variants into: Dukes A cancer, confined to the bowel wall in the original classification and does not penetrate muscularis propria; Dukes B classification refers to a tumour that has penetrated the muscle wall and possibly invaded the pericolic fat but did not metastasise to lymph nodes; Dukes C tumour is similar to Dukes B but shows lymph node metastases: Dukes D (added after establishment of Dukes classification) signifies distant metastases. The TNM classification is replacing the Dukes classification. T1 tumour invades submucosa, T2 tumour invades muscularis propria, T3 tumour invades through muscularis propria into subserosa or into pericolic or perirectal tissues and T4 when tumour directly invades other organs and structures and/or perforates visceral peritoneum.

Normal colon tissues

The specimens of colon of nine patients operated for diverticulosis (without inflammation) served as normal colon tissue representatives. Specimens of the colon mucosa taken at least 15 cm from all carcinoma samples were also used as references of the non-malignant colon tissue in carcinoma patients. For all of them histopathological examination showed the absence of tumour cells.

Tissue processing

All tumours used in the study were surgically or endoscopically resected in Department of Abdominal Surgery or Department of Gastroenterology at Clinical

Hospital Centre Zagreb. The specimens were fixed in 10% buffered formalin immediately after resection, dehydrated in ethanol and embedded in paraffin. Representative paraffin blocks of each tumour were cut in two 5 μ thin slices, deparaffinated and dehydrated on glass slides for immunohistochemistry. One slide of each tumour was stained with hematoxylin and eosin (Kemika, Croatia) and other prepared for immunohistochemistry with monoclonal antibody (mAb) against acrolein. The patients were not treated with any drug during the time from diagnoses up to the time of surgery. Two registered pathologist (Zarkovic and Hlupic) diagnosed each specimen independently.

Monoclonal antibodies against acrolein-modified proteins

Anti-acrolein-modified protein monoclonal antibody (5F6) was generated as previously described by Uchida et al. [22]. The specificity study has shown that, among the aldehydes tested, acrolein was the only source of antigenic materials generated in the protein [22].

Immunohistochemistry

For immunohistochemical staining, one slice of each tumour was cut into 5 μ thin sections, mounted on the slide coated with 3-amino-propyl-triethoxy silane, deparaffinated in xylene and rehydrated trough a series of ethanol. Immunohistochemistry was done in a three step procedure (LSAB kit, Dako, Denmark) where the first step was incubation with anti-acrolein-modified KLH mAb5F6 during 2 h in humid chambers at room temperature. The second step was incubation with biotinylated secondary goat anti-mouse and anti-rabbit immunoglobulins (AB2) during 30 min. The third step was incubation with streptavidin peroxidase (HRP) during 30 min. Finally, the reaction was visualised by a DAB + (3,3, -diaminobenzidine tetrahydrochloride in organic solvent) during 10 min. Competition experiments to confirm the specificity of immunostaining were performed with the antibody that was preincubated for 4 h at 37°C with an excess of N^{α}-acetyl-FDP-lysine. Non-immune mouse IgG was used as a negative control.

The same procedure was applied also to detect p53 distribution in the tissues using monoclonal mouse anti-human p53 (Clone DO-7, DAKO, Denmark), which reacts with both the wild-type and mutant forms of this protein. For the p53 immunodetection, the specimens were pretreated by citrate buffer (pH 6.0) incubation in microwave oven.

Intensity and distribution of acrolein and p53 immunostaining in the tumour and the surrounding nontumourous mucosa were evaluated semiquantitatively. The absence of immunopositivity in epithelial cells was marked with negative sign (−), while the +/− was assigned to the samples showing occasional positivity in some cells only. With one cross (+) we marked weak immunopositivity in less then 25% of the epithelial cells, with two crosses (++) medium immunopositivity in 25–50% of cells, and with tree crosses (+++) strong immunopositivity in more then 50% cells. In soft tissues of the gut and stromal elements of the tumour we distinguished only positive (+) and negative (−) immunostaining.

Statistical analyses

The incidence of acrolein positive vs. acrolein negative tissues depending on the type of tumours was evaluated by Chi-square test. Possible differences in intensity of staining was done by Mann-Whitney test, using numerical description of positivity corresponding to respective standard grading of positivity as described above.

Results

Intensity and distribution of acrolein in tumours and in nontumourous colon tissue was studied (Figure 1). Weak (+) acrolein immunostaining was present in only 4 of 24 tubular adenomas (Table I). Immunoreactivity was confined to cytoplasm of epithelial tumour cells and was absent in nucleus. In half (12/24) of analysed tubular adenomas stroma was acrolein-positive while positivity was also noticed in the infiltrating leukocytes.

In 10 of 24 low-grade tubulovillous adenomas weak to medium acrolein immunoreactivity was noticed (Table II). It was restricted to cytoplasm and was not found at all in nuclei (0/24) of analysed tumours. The immunostaining in tumour stroma (14/24) and infiltrating lymphocytes (16/24) were present as in tubular adenomas. In high-grade villotubular adenomas (Table III) the incidence (14/20) of acrolein positive tumours was higher ($p = 0.01$) than in the low-grade variant (10/24). Immunopositivity was of

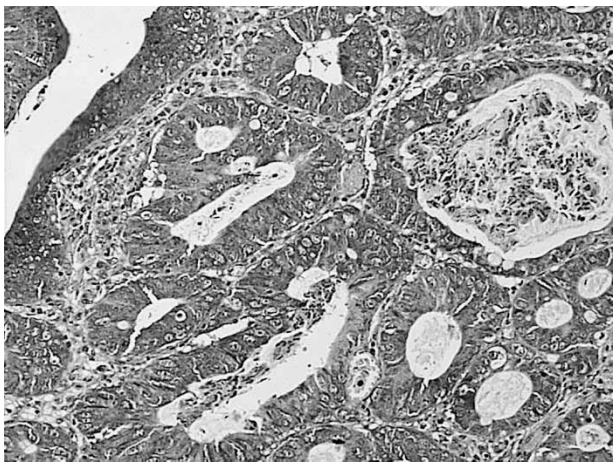

Figure 1. Immunohistochemical appearance of acrolein–protein adducts in Dukes A carcinoma. Most of tumour cells show immunopositivity for acrolein–protein adducts in cytoplasm, while their nuclei are negative (transparent). DAB staining (as described in "Materials and methods" section), magnification 400 × .

Table I. Distribution of acrolein in tubular adenomas.

Patient no.	Tumor diameter (cm)	Patient age (years)	Patient sex	Acrolein immunoreactivity			
				Epithelium cytoplasm	Epithelium nucleus	Stroma	Inflammatory cells in stroma
1	1.5	67	m	−	−	−	−
2	2.0	44	m	−	−	+	+
3	0.2	59	m	−	−	−	−
4	0.2	59	m	−	−	+	−
5	1.5	72	m	−	−	+	+
6	1.5	72	m	+	−	+	+
7	0.3	63	F	−	−	−	+
8	0.5	60	m	+	−	−	−
9	1.8	43	m	−	−	+	+
10	1.8	43	m	+	−	−	+
11	0.4	74	m	−	−	+	+
12	0.5	72	F	−	−	+	−
13	0.5	72	F	−	−	+	−
14	0.5	76	F	−	−	+	−
15	0.4	69	m	−	−	+	−
16	0.7	68	m	−	−	−	−
17	0.6	68	F	−	−	−	+
18	0.6	56	m	−	−	−	+
19	0.4	48	F	−	−	+	+
20	0.6	65	m	−	−	+	+
21	0.4	77	F	+	−	−	+
22	0.6	69	F	−	−	−	+
23	0.6	60	m	−	−	−	+
24	0.4	56	F	−	−	−	+

Table II. Distribution of acrolein in low-grade villotubular adenomas.

Patient no.	Tumor diameter (cm)	Patient age (years)	Patient sex	Acrolein immunoreactivity			
				Epithelium cytoplasm	Epithelium nucleus	Stroma	Inflammatory cells in stroma
1	0.5	79	m	−	−	+	+
2	1.2	56	m	−	−	+	+
3	0.5	71	m	+	−	−	+
4	3.5	53	f	+	−	−	−
5	3.5	53	f	−	−	−	−
6	3.5	53	f	++	−	−	−
7	3.5	53	f	+	−	−	−
8	3.5	53	f	−	−	−	+
9	0.6	68	m	−	−	+	+
10	1.0	49	m	++	−	−	−
11	1.5	61	f	+	−	+	+
12	0.5	67	m	−	−	+	+
13	0.5	72	f	+	−	+	−
14	0.8	57	m	++	−	+	+
15	1.8	81	f	+	−	−	−
16	1.8	81	f	−	−	++	+
17	0.5	72	f	+	−	+	+
18	0.7	69	f	−	−	−	+
19	0.5	43	m	−	−	++	−
20	1.5	65	m	−	−	−	+
21	0.7	79	m	−	−	+	+
22	0.8	79	m	−	−	+	+
23	0.8	79	m	−	−	+	+
24	0.7	58	m	−	−	+	+

Table III. Distribution of acrolein in high-grade vilotubular adenomas.

Patient no.	Tumor diameter (cm)	Patient age (years)	Patient sex	Acrolein immunoreactivity			
				Epithelium cytoplasm	Epithelium nucleus	Stroma	Inflammatory cells in stroma
1	4.5	56	f	++	−	++	−
2	4.5	56	f	+	+	++	−
3	1.2	61	m	−	+	++	+
4	5.0	69	m	+	−		+
5	1.0	67	m	−	++	+++	++
6	1.0	67	m	−	++	++	++
7	1.2	49	m	+	−		−
8	2.0	60	f	+	−	+++	−
9	1.5	61	f	−	−		−
10	3.5	61	f	++	−	+	−
11	0.9	62	m	+	−	+	−
12	0.9	62	m	+	−	++	−
13	1.5	87	m	+	−	+	−
14	1.8	45	f	−	−		−
15	3.0	59	m	+	−		+
16	3.0	59	m	++	−	++	+
17	3.0	61	f	+	+	+	+
18	4.0	73	f	+	−	+	−
19	2.0	52	m	−	−		+
20	0.9	59	f	++	−	+	−

weak to medium intensity and present mostly in the cytoplasm of epithelial tumour cells. However, in high-grade villotubular adenomas acrolein positivity was also noticed in nuclei of some tumours (5/20). In adenocarcinomas weak acrolein immunostaining in cytoplasm of epithelium was present in almost all analysed cases. Frequent presence of similar intensity of positive reaction was also found in stromal structures, submucosa, subserosa and adipose tissue, as well as in stromal leukocytes (Tables IV–VI). The aldehyde was only sporadically noticed in smooth muscle layer, irrespective of the carcinoma stage (data not presented). However, there were obvious differences in acrolein presence in malignant cells, depending on the carcinoma stage (Tables IV–VI, Figure 2).

Table IV. Distribution of acrolein in Dukes A carcinomas.

Patient no.	Tumor diameter (cm)	Patient age (years)	Patient sex	Acrolein immunoreactivity			
				Epithelium cytoplasm	Epithelium nucleus	Stroma	Inflammatory cells in stroma
1	2.5	76	m	−	+	+	+
2	10.0	21	f	−	++	+	+
3	6.0	73	m	−	+++	−	++
4	3.0	55	m	−	++	+	−
5	4.5	51	f	−	+	+	−
6	4.5	52	f	+	+	+	−
7	5.0	83	m	−	++	+	+
8	5.0	68	m	++	−	+	+
9	5.0	72	m	++	−	+	+
10	7.0	64	m	++	−	+	−
11	6.0	72	m	++	−	−	−
12	5.5	81	m	+	+	−	−
13	5.0	78	f	+++	−	+	−
14	3.3	55	f	++	−	+	−
15	7.0	60	f	++	−	+	+
16	3.4	62	m	++	−	+	+
17	4.0	66	m	+	−	−	−
18	5.0	67	f	+	−	−	+
19	3.0	69	m	+	−	−	+

Table V. Distriution of acrolein in Dukes B carcinomas.

Patient no.	Tumor diameter (cm)	Patient age (years)	Patient sex	Acrolein immunoreactivity			
				Epithelium cytoplasm	Epithelium nucleus	Stroma	Inflammatory cells in stroma
1	5.3	47	f	−	−	+	+
2	5.0	49	f	+	++	+	+
3	5.5	63	f	+	−	+	+
4	7.0	62	m	+	−	+	+
5	3.0	88	m	−	−	+	−
6	5.0	64	f	−	+	−	−
7	4.0	61	m	−	−	−	−
8	9.0	69	m	−	−	−	−
9	10.0	54	m	−	−	−	+
10	5.5	71	m	+	−	−	+
11	8.0	62	m	+	−	−	+

The Dukes A group comprised 19 carcinomas and they were all positive to acrolein (Table IV). Eight of them corresponded to T1 grade and eleven to the T2 grade. Twelve of 19 Dukes A adenocarcinomas showed medium degree of acrolein immunostaining in the cytoplasm, which was equally distributed between T1 (6/8) and T2 (6/11) grade tumours ($p = 0.18$). Nuclear acrolein presence was noticed in 8 of 19 Dukes A tumours: in one quarter (2/8) of T1 grade tumours and in more then half (6/11) T2 grade tumours ($p = 0.05$).

Dukes A adenocarcinomas had acrolein immunopositive staining present either in cytoplasm or in nuclei, while simultaneous positivity was noticed only for two tumours. Acrolein immunostaining in stroma and infiltrating lymphocyte in the tissue of T1 grade tumours was similar to high-grade villotubular adenomas (Tables III and IV). However, intensity of acrolein positivity was slightly stronger in altered epithelium of Dukes A carcinomas than in high-grade villotubular adenomas ($p = 0.027$). In carcinomas of T2 grade acrolein was detected clearly in nuclei while cytoplasmic immunostaining appeared less obvious than observed for T1 grade tumours (Table IV). In Dukes B group were 11 tumours and all of them corresponded to T2 grade adenocarcinomas (Table V). Five of 11 tumours had weak acrolein immunopositivity in cytoplasm of carcinoma cells and in stroma. In only 2 of 11 carcinomas nuclei of tumour cells were immunopositive for acrolein. Thus, the acrolein immunostaining was significantly lower ($p = 0.001$) in Dukes B group when compared with the Dukes A malignancies.

Dukes C group comprised 15 adenocarcinomas: 10 of them corresponded to T3 grade and 5 to T4 grade (Table VI). In one thread (5/15) of Dukes C adenocarcinomas cytoplasm of tumour cells was moderately positive to acrolein. Four of five adenocarcinomas positive for acrolein were T3 and

Table VI. Distribution of acrolein in Dukes C carcinomas.

Patient no.	Tumor diameter (cm)	Patient age (years)	Patient sex	Acrolein immunoreactivity			
				Epithelium cytoplasm	Epithelium nucleus	Stroma	Inflammatory cells in stroma
1	4.5	69	m	+	+	++	+
2	6.0	57	m	+	−	−	−
3	6.0	64	f	−	−	++	−
4	1.8	62	f	+	−	+	−
5	3.5	68	f	−	−	−	−
6	10.0	43	m	−	−	+	−
7	3.0	50	m	+	−	+	+
8	5.0	66	f	−	−	+	−
9	4.0	66	m	+	+	+	−
10	4.5	69	f	−	−	+	+
11	3.0	57	f	−	−	−	−
12	4.0	53	m	−	−	+	−
13	5.0	72	f	−	−	−	+
14	5.5	78	f	−	−	−	−
15	4.0	57	m	−	−	−	−

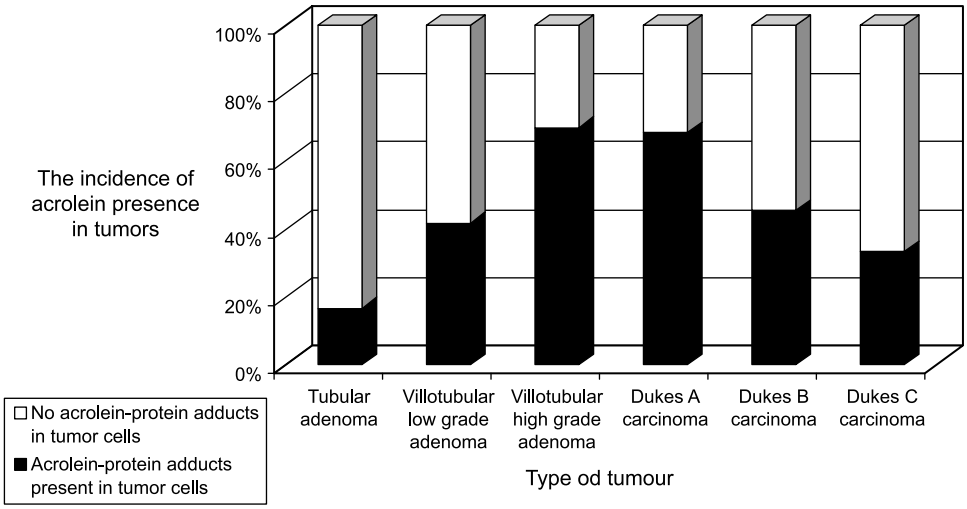

Figure 2. The incidence of acrolein–protein adducts in colon tumours. Number of tumours samples analysed: tubular adenomas—24, low-grade villotubular adenomas—24, high-grade villotubular adenomas—20, Dukes A carcinomas—19, Dukes B carcinomas—11, Dukes C carcinomas—15.

only one was T4 grade. Weak nuclear positivity was observed in 2/15 carcinomas. Stroma showed equal incidence ($p > 0.1$) of weak acrolein immunostaining in both T3 (6/10) and T4 (3/5) tumours in Dukes C group. The same was also intensity of acrolein staining for Dukes C and B carcinomas ($p > 0.1$), while Dukes C carcinoma cells had weaker acrolein expression than malignant cells in Dukes A carcinomas. Thus, the overall distribution of acrolein appearance in colon tumours summarised in Figure 2 shows prevalence of acrolein–protein adducts in high-grade villotubular adenomas and Dukes A carcinomas in the overall 113 tumours analysed. In comparison to tubular adenomas, low-grade villotubular adenomas and especially high grade villotubular adenomas have shown significantly higher incidence of acrolein positivity in tumour tissue ($p < 0.001$). High grade villotubular adenomas had the same incidence of acrolein positivity as Dukes A adenocarcinomas ($p > 0.1$), while acrolein positivity was gradually decreasing with tumour progression, so that Dukes C carcinomas had significantly lower incidence of acrolein positivity than Dukes A carcinomas ($p = 0.003$).

The presence of acrolein protein adducts in non-malignant colon tissues of carcinoma patients and normal colon are presented in Table VII. All

Table VII. The presence of acrolein − protein adducts determined by immunohistochemistry in non-malignant colon tissues.

	The presence of acrolein in non-malignant colon epithelium of patients with diagnosis			
Patient no.*	Diverticulosis**	Dukes A carcinoma	Dukes B carcinoma	Dukes C carcinoma
1	+	++	++	+++
2	++	+	+	++
3	−	++	+	++
4	+	++	++	++
5	−	+	+	+++
6	−	+	+	+++
7	−	+	++	+++
8	−	++	+	+++
9	+	+++	+	+++
10		++	+	+++
11		+++	+	++
12		+		+++
13		++		+++
14		+		++
15		+		+++
16		+		+++
17		+		+
18		+		++
19		++		+++

*According to the Tables I–VI. **Tissue without any pathological process.

non-malignant colon tissue samples of carcinoma patients were positive for acrolein. Although, acrolein was detected also in normal colon epithelium in 4/9 patients in comparison to normal colon (diverticulosis) non-malignant colon tissues of carcinoma patients showed much stronger acrolein presence (for all $p < 0.05$). The strongest acrolein expression was detected for Dukes C carcinomas, which was stronger than in Dukes B or A carcinomas (for both, $p < 0.001$).

The p53 was not detected in non-malignant colon epithelium, while it was pronounced in carcinomas, in particular in advanced stages (data not presented).

Discussion

The results obtained show that in colon carcinoma patients acrolein–protein adducts are abundant in non-malignant colon tissue, remote from malignancy. Intensity of acrolein presence increases also in colon adenomas and carcinomas, being the most prominent in high-grade villotubular adenomas and carcinomas of an early stage, i.e. Dukes A.

While acrolein was found to be mostly conjugated to cytoplasmatic proteins in Dukes A, particularly in T1 carcinomas, the presence of acrolein was also found often in the nuclei of malignant cells. Advanced stages of carcinomas (Dukes B and C correspond to T2, T3 and T4) were again associated with less obvious acrolein presence in the tissues, although in non-malignant colon epithelium of these patients acrolein was abundant.

This resembles previous findings on the presence of another reactive aldehyde—HNE in colon carcinomas of different stage and its interference with expression of TGF-β1 and its type I and II receptors [25]. Hence, dissregulation of lipid peroxidation and the cytokine growth control seems to be associated with de-differentiation of normal colon epithelium and eventually malignant alteration of high-grade villotubular adenomas into Dukes A (T1 and T2) colon adenocarcinomas. Higher cytoplasmic and nuclear acrolein positivity in high-grade villotubular adenomas and Dukes A colon cancer (particularly in T1 grade tumours) than in advanced cancer Dukes B and C suggest importance of oxidative stress and lipid peroxidation in colon carcinogenesis, i.e. transition from benign into malignant colon tumours. The support for our results suggesting carcinogenic effects of acrolein are also immunohistochemical data that showed formation of acrolein-derived 2′-deoxyadenosine adducts in an iron-induced carcinogenesis model in kidneys of rodents [26]. High levels 8-OHdG were found in various human cancers *in vivo*, whereas, they were low in human cancer cell lines *in vitro*, suggesting caution in studies evaluating the carcinogenic effects of oxidative stress *in vitro*, in particular molecular mechanisms involved in complex spread of harmful events between different types of cells [27]. The differences between high levels of 8-OHdG found in cancer cells *in vivo* and low values in human cancer cell lines *in vitro*, might be in a part due effects of stromal cells *in situ*, in particular stromal inflammatory cells. These cells produce large amounts of ROS generating lipid peroxidation such as HNE and acrolein and consequently 8-OHdG and the aldehyde-protein as well as the aldehyde-DNA adducts [16]. In favour of this possibility is the fact that positivity for acrolein in tumour stroma and its inflammatory cells resembled the presence of acrolein in tumour cells, also depending on the type of tumour and its stage.

Furthermore, while HNE is also chemoattractant, acrolein prevents apoptosis of neutrophils through inhibition of caspase-3 [28]. Hence, acrolein may subsequently increase neutrophil recruitment initiated by HNE and thus increase oxidative stress due to the oxidative burst of leukocytes in the affected tissue. This may further lead to circulus viciosus generating the reactive aldehydes which can be only partly eliminated and will therefore cause either lethal or carcinogenic damages [28,29]. Eventually, malignant tissues will consist mostly of cancer cells with high turnover of lipids and reduced amounts of PUFAs, which will result in reduction of acrolein in the tissue, after the critical damages to DNA and proteins caused their carcinogenic effects. This might offer explanation for reduced acrolein and HNE in advanced carcinomas in comparison to early stage carcinomas.

Acrolein is among α,β-unsaturated aldehydes the one that has the highest reactivity toward proteins and DNA [22,30]. Thus, it is not surprising that we noticed its presence even in non-malignant cells. It is possible that intestinal acrolein is generated by the colon tissue or inflammatory cells, but it could also be of exogenous, dietary origin. A possibility of acrolein arising from dietary aldehydes or lipid peroxides seems very important [31,32]. The diets rich in fat and low in antioxidants could thus be relevant source of the aldehyde's presence in non-malignant colon tissues and could also influence the aldehyde's association with colon carcinogenesis.

However, it has yet to be evaluated if acrolein has some physiological role, as does HNE, which acts as a signalling molecule in the regulation of the cell growth control [20,33–35]. One of the important effects of HNE is that after it binds to glutathione (GSH) causing a transient decrease of this major antioxidant peptide, synthesis of GSH is elevated through induction of glutamate cysteine ligase (GCL), which catalyses the first step in *de novo* synthesis of GSH [36]. Consequently, increased synthesis of GSH leads to elimination of HNE. Acrolein is also removed by GSH, so it is also able to reduce rapidly cellular GSH content, in particular in mitochondria, but there is no evidence that it could also increase GSH synthesis [37,38].

Hence, these particular products of lipid peroxidation could interact both under physiological and pathological conditions as an integral part of systemic cell growth control through autorcine/paracrine mechanisms, with possible differential effects on malignant and on respective non-malignant cells, which will depend on the formation of their protein adducts [33–41]. In this respect, it is important to say that we did not notice any relationship between the presence of p53 and acrolein in tumours or non-malignant colon tissues. We found p53 increased in advanced colon carcinomas, which is a well known parameter of the colon carcinoma progression [2], while we found acrolein to be less produced in advanced carcinomas than in early stage tumours. On the contrary, acrolein–protein adducts were increased in non-malignant colon tissues of carcinoma patients, while p53 was not detected in these tissues at all. Accordingly, we must conclude that possible carcinogenic effects of acrolein in colon tumours are not associated with p53 expression.

Thus, molecular mechanisms of possible carcinogenic effects of acrolein in transition from normal to malignant tumours and the spread of oxidative stress from malignant in adjacent non-malignant tissues require further studies. This may have additional impact in oncology because acrolein is a metabolite of allyl compounds biotransformation and also of the widely used anticancer drug cyclofosphamide [21]. However, because our patients did not receive any chemotherapy before surgical removal of the tumours, development of acrolein in non-malignant colon tissues of carcinoma patients was certainly not caused by any kind of chemotherapy.

Conclusion

This study defines involvement of acrolein in colon carcinogenesis, shows involvement of lipid peroxidation in malignant transition of tumours from adenoma into carcinoma and the spread of lipid peroxidation from malignant into adjacent non-malignant colon tissue, confirming the relevance of acrolein–protein adducts as bioactive marker of lipid peroxidation.

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