Research Article

TumorBiology

Tumor Biol 2006;27:235–242
DOI: 10.1159/000094694

Received: August 30, 2005
Accepted after revision: December 8, 2005
Published online: July 19, 2006

Nucleosomes in Colorectal Cancer Patients during Radiochemotherapy

Andreas Kremer[a] Stefan Holdenrieder[a] Petra Stieber[a] Ralf Wilkowski[b] Dorothea Nagel[a] Dietrich Seidel[a]

[a]Institute of Clinical Chemistry and [b]Institute of Radiotherapy and Radiooncology, Klinikum der Universität München – Grosshadern, Munich, Germany

Key Words

Nucleosomes · DNA · Apoptosis · CEA · CA 19-9 · CYFRA 21-1 · Tumor marker · Colorectal cancer · Radiochemotherapy

Abstract

Apoptotic markers and tumor-associated antigens might be suitable to indicate the response to radiochemotherapy early. We analyzed the courses of nucleosomes, CEA, CA 19-9 and CYFRA 21-1 in 25 colorectal cancer patients during radiochemotherapy (4 postoperative, 13 preoperative, 8 local relapse therapy). Blood was taken before therapy, daily during the first week, once weekly during the following weeks, and at the end of the radiochemotherapy. After a temporary decline 6 h after the first irradiation, nucleosomes rose in most patients rapidly reaching a maximum during the first days which was followed by a subsequent decrease. In patients receiving postoperative therapy after complete resection of tumor, nucleosome levels generally were lower than in patients with preoperative or relapse therapy. Correspondingly, CEA, CA 19-9 and CYFRA 21-1 levels of postoperatively treated patients were the lowest whereas those with tumor relapse had the highest ones. During preoperative therapy, lower nucleosome concentrations were found in patients with response to therapy resulting in a smaller area under the curve of days 1–3 (AUC) than in those with progressive disease ($p = 0.028$). The other parameters did not indicate the response to therapy at the initial treatment phase. In conclusion, the course of nucleosomes (AUC) might be valuable for the early prediction of therapy response in preoperatively treated colorectal cancer patients.

Introduction

Colorectal cancer ranks as the third most common cancer in men and women. It is the second leading cause of cancer death in the United States [1]. Besides surgery, therapeutic options include radiotherapy, chemotherapy and also a combination of both. In these systemic modalities, apoptosis is one of the main mechanism leading to the demise of cancer cells.

Nucleosomes, which are complexes formed by an octamer of histones with DNA twisted around, are typical products of apoptotic cell death [2, 3]. They are the basic elements of chromatin that is, during apoptosis, cleaved into mono- and oligonucleosomes by endonucleases [4, 5]. These particles are packed into apoptotic bodies and

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1010–4283/06/0275–0235$23.50/0

Accessible online at:
www.karger.com/tbi

Petra Stieber
Klinikum der Universität München – Grosshadern, Marchioninistrasse 15
DE–81377 München (Germany)
Tel. +49 89 7095 3115, Fax +49 89 7095 6298
E-Mail Petra.Stieber@med.uni-muenchen.de

Table 1. Characteristics of the patients investigated

	Postoperative RCT (n = 4)	Preoperative RCT (n = 13)		Relapse RCT (n = 8)
		No Prog. (n = 9)	Prog. (n = 4)	
Age (median, range), years	65 (54–75)	64 (47–76)		64 (47–73)
Gender (female/male)	2/2	3/10		0/8
Stage				
UICC I	0	0	0	1
UICC II	3	4	1	1
UICC III	1	4	3	3
UICC IV	0	1	0	2
Pathological differentiation				
Well differentiated	0	0	0	0
Moderately differentiated	3	9	1	6
Poorly differentiated	1	0	2	1
Without classification	0	0	1	1

RCT = Radiochemotherapy; No Prog. = no progression; Prog. = progression.

are phagocytized by neighboring cells and macrophages. In case of enhanced cell death, such as during radio- and chemotherapy, nucleosomes are also released into circulation [6, 7], and are detected in elevated concentrations in serum and plasma.

Circulating nucleosomes can be quantified directly by ELISA techniques [8]. Previous investigations showed low levels of nucleosomes in the serum of healthy individuals [9]. In patients with various malignant tumors high amounts of nucleosomes appear spontaneously and under chemo- and radiotherapy [10, 11]. Elevated nucleosome levels are also observed in benign pathological conditions like infections [12], autoimmune diseases [13, 14], graft-versus-host reactions [15], trauma [16], stroke [17, 18], and exhaustive exercise [19].

As the course of nucleosomes correlated with the response to radiotherapy and chemotherapy in patients with various cancers, they showed potential for the monitoring of systemic therapies [12, 20]. Additionally recent results have revealed that the initial course of nucleosomes during radiochemotherapy was a significant indicator for the time to progression in patients with pancreatic cancer [21]. Further in patients with advanced lung cancer, nucleosomes were able to predict the response to chemotherapy already after the first application independently from clinical factors and CYFRA 21-1 [22].

As in the present study on patients with colorectal cancer radiochemotherapy was applied in some patients after surgical resection of the primary tumor, in others preoperatively to reduce tumor volume before resection, and in others who suffered from local tumor relapse, we first investigated whether the quantity of the marker release was associated with the presence of tumor burden during therapy. Further, we asked whether the courses of nucleosomes and of other tumor-associated antigens during the initial phase of radiochemotherapy are able to predict the therapy response in colorectal cancer patients early.

Patients and Methods

Patients

In total, 25 patients with colorectal cancer were included in this study. All patients were treated with a combined radio- and chemotherapy. Among them were 4 with postoperative therapy after complete resection of tumor, 13 with preoperative therapy, and 8 with local relapse therapy. All patients were investigated pretherapeutically by coloscopy, biopsy, and computed whole-body tomography. Pathological grading was established in 23 patients, with 19 patients having a moderate and 4 a poor differentiation grade. All but 1 patient had adenocarcinoma. Involvement of lymph nodes was proven in 1 patient of the postoperative group, while 3 patients were free of malignant lymph nodes. Seven patients of the preoperative and relapse group were classified as nodal negatives, 13 as nodal positives (table 1).

Treatment planning and field positioning were performed individually by 3D calculation (HELAX) and CT scans. Radiation was delivered in a three- or four-field technique using a linear accelerator with a photon beam of more than 10 MV. The treated volume included the macroscopic tumor with a safety margin of 2–3 cm, the perirectal and internal iliac lymph nodes. The anus was only irradiated in case of malignant infiltration of the anal canal. A single dose of 1.8 Gy was applied on 5 days a week. Postoperatively

and preoperatively treated patients received 25–30 fractions within 5–6 weeks. Thus, the total dose was 45.0–54.0 Gy (dose rate according the International Commission of Radiation Units and Measurements, ICRU 50). Patients with local relapse of tumor received 17 fractions (reirradiation), which equals a total dose of 30.7 Gy.

5-Fluorouracil (500 mg/m^2 per day) was additionally given as a continuous 5-day infusion on weeks 1 and 5 for the postoperative treatment protocol. Patients receiving preoperative and relapse therapies received daily infusions of 5-fluorouracil (350 mg/m^2).

Blood was taken before the start of therapy, 6 h after the first radiation, daily during the first week, once weekly during the following weeks and at the end of radiochemotherapy.

In patients treated preoperatively, staging was done before the start of radiochemotherapy, in most cases by computed whole-body tomography. Response to therapy was determined by postsurgery pathological staging within 2 months after the end of therapy. In our evaluation, all patients having a downstaging of the tumor or alternatively 'stable disease' were considered as 'no progression'. They were compared with patients suffering from progressive disease.

The study was approved by the local ethics committee. Before inclusion in the study, written informed consent was given by all patients.

Methods

Nucleosome concentrations were determined in serum samples. Within 1–2 h after venipuncture the blood samples were centrifuged at 3,000 *g* for 15 min. Subsequently, 10 m*M* EDTA was added as stabilizer and the serum samples were stored at –70°C. Prior to the measurement of nucleosomes, samples were thawed, homogenized and diluted 1:4 with an incubation buffer.

For the quantification of nucleosomes concentrations in serum the Cell Death Detection ELISA^{plus} (Roche Diagnostics) was used. In this assay, two monoclonal mouse antibodies directed against histones and DNA catch the nucleosomes specifically. The anti-histone antibody binds to the microtiter plate, whereas the anti-DNA antibody, which is labeled with peroxidase, reacts with 2,2′-azino-di(3-ethylbenzthiazolin-sulfonate). The amount of nucleosomes captured by the antibodies is proportional to the resulting color development and enables the photometrical quantification.

The tumor-associated antigens CEA, CA 19-9 and CYFRA 21-1 were determined in all samples by Elecsys 2010, Roche Diagnostics, Germany.

Statistics

For our evaluation we considered the pretherapeutic concentrations, the levels of day 1 (6 h after the first application of the therapy), day 2 and day 3 of all parameters. The values during the initial phase of therapy were summarized by the area under the curve of days 1–3 (AUC) which includes the concentrations before (day 1, 0 h), 6 h (day 1, 6 h), 24 h (day 2), and 48 h (day 3) after the start of therapy, divided by the number of days. Generally, results are presented as medians and ranges.

An overall analysis of variance was performed to test the general dependency of logarithmical concentrations of nucleosomes, CEA, CA 19-9 and CYFRA 21-1 on the treatment group (postoperative, preoperative and relapse therapy) and on time [before (day 1, 0 h) and after (day 1, 6 h to day 3) start of radiochemotherapy]. This was done using the SAS procedure MIXED, which can take into account dependencies of repeated values of the same patient. In addition, adjusted p values were calculated according to the number of comparisons between the three treatment groups.

In the same way marker concentrations in patients with preoperative radiochemotherapy having 'no progression' and 'progression' of disease were compared. Additionally the Wilcoxon test was used to compare the AUC between both groups. The comparison of patients with progression and without progression is shown as dot plots.

A p value <0.05 was considered statistically significant. All calculations were performed by software of SAS (version 8.2, SAS Institute, Cary, N.C., USA).

Results

Generally, most of the patients showed a temporary decrease of the nucleosome concentration 6 h after the first irradiation followed by a considerable and rapid increase with a maximum during the following days. Subsequently, the levels declined again. All patients receiving postoperative radiochemotherapy after complete resection of the primary tumor released only low levels of nucleosomes before and during the time of radiochemotherapy. The initial peak mostly was only little pronounced (fig. 1a). Patients receiving preoperative therapy showed considerably higher nucleosome levels and higher initial peaks during radiochemotherapy compared to postoperatively treated patients. Within the preoperative group, those with response to therapy had lower nucleosome concentrations than those with progressive disease (fig. 1b, c).

Between patients receiving postoperative therapy and those with preoperative and relapse therapy, significant differences were observed concerning nucleosomes and other tumor-associated antigens. When the primary tumor was removed before radiochemotherapy, the marker concentrations were notably lower than in the presence of tumor burden, particularly for nucleosomes and also for CEA and CYFRA 21-1. Additionally, the levels of the tumor-associated antigens CEA, CA 19-9 and CYFRA 21-1 were further enhanced in patients who suffered from tumor relapse compared to those with a primary tumor. The detailed results are summarized in table 2.

In patients with primary advanced colorectal cancer, preoperative radiochemotherapy was applied to reduce the tumor volume. Concerning the change of tumor stage according to the UICC classification at the time of the preoperative restaging investigation, 4 patients showed remission, 5 patients had stable disease and 4 patients

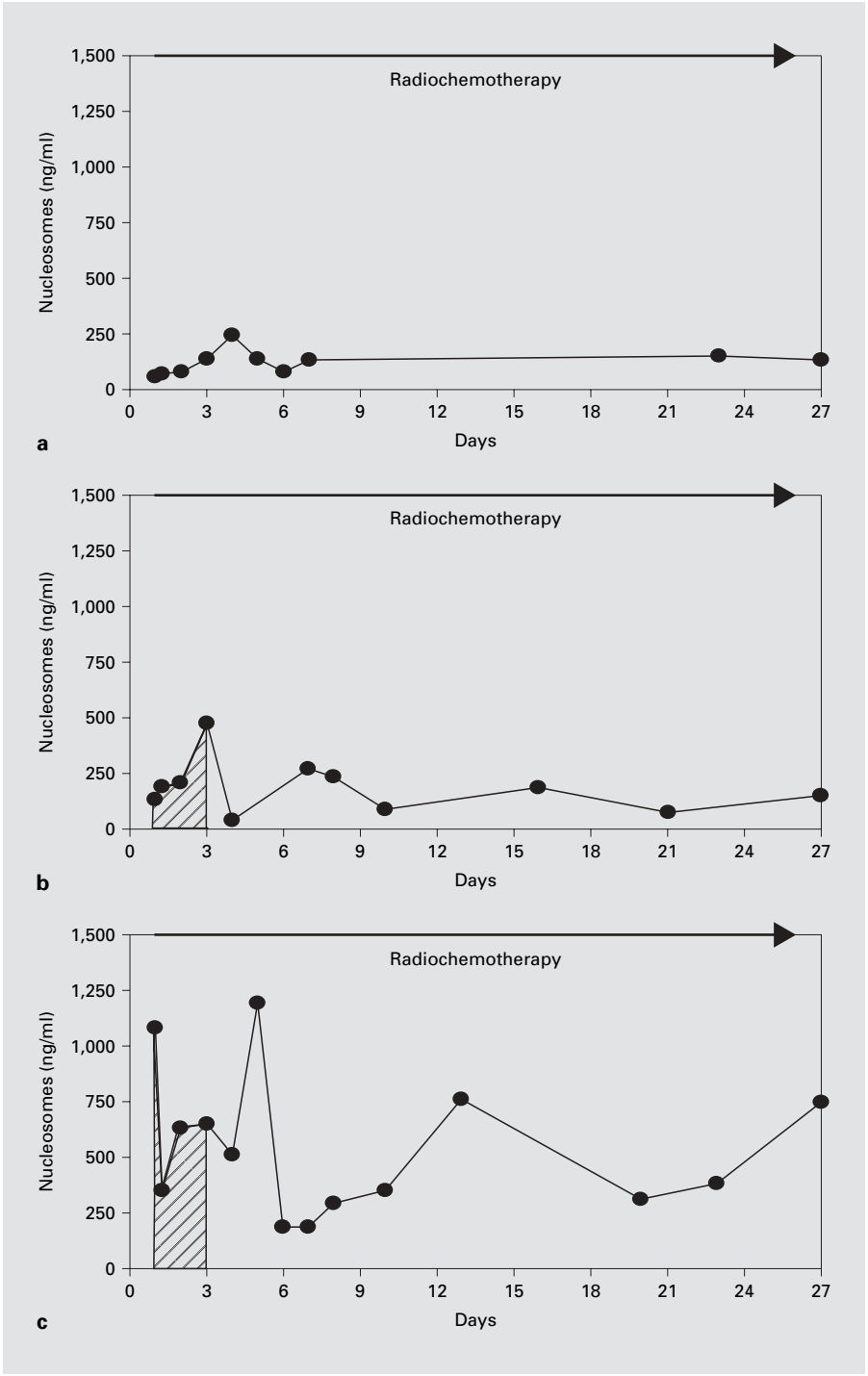

Fig. 1. Characteristic courses of nucleosomes in serum of a patient with rectal cancer (pT3, pN0, M0, G3) during radiochemotherapy after complete resection of tumor (**a**), a patient with rectal cancer (T3, N0, M0, G2) during preoperative radiochemotherapy without progression of disease (**b**), and a patient with rectal cancer (T3, N1, M0, G3) during preoperative radiochemotherapy with progressive disease (**c**).

suffered from progression. When combining patients with remission and stable disease in the group of 'no progression', they had a significantly ($p = 0.028$) smaller AUC compared to those with progressive disease (fig. 2). The biological markers CEA, CA 19-9 and CYFRA 21-1 could not discriminate between the nonprogressive and the progressive group (table 3).

Table 2. Medians and ranges of nucleosomes, CEA, CA 19-9 and CYFRA 21-1 in patients receiving postoperative, preoperative and relapse radiochemotherapy concerning the pretherapeutic value (day 1, 0 h), the values 6 h after the start of therapy (day 1, 6 h) and during the following days (day 2; day 3)

	Nucleosomes ng/ml	CEA ng/ml	CYFRA 21-1 ng/ml	CA 19-9 U/ml
Postoperative RCT (n = 4)				
Day 1, 0 h	192 (86–530)	2.0 (0.8–3.6)	0.9 (0.5–1.4)	11.1 (4.3–13.0)
Day 1, 6 h	126 (78–424)	1.1 (0.7–3.7)	1.3 (0.6–1.5)	8.6 (4.1–10.4)
Day 2	208 (45–274)	1.7 (0.8–3.2)	1.1 (0.6–1.1)	9.5 (4.4–11.9)
Day 3	238 (135–464)	1.9 (1.2–3.0)	1.1 (0.6–1.2)	8.9 (8.9–9.9)
Preoperative RCT (n = 13)				
Day 1, 0 h	1,154 (238–4,422)	4.6 (1.5–43.3)	1.7 (0.6–39.4)	11.8 (4.0–65.4)
Day 1, 6 h	502 (145–957)	7.3 (1.4–45.2)	1.5 (0.9–4.9)	11.3 (3.7–59.7)
Day 2	732 (370–1,329)	5.0 (1.5–45.5)	2.0 (0.9–36.9)	11.0 (3.9–47.1)
Day 3	879 (174–2,365)	2.7 (1.5–41.7)	1.9 (1.0–4.8)	10.8 (3.7–52.6)
Relapse RCT (n = 8)				
Day 1, 0 h	993 (443–3,767)	17.3 (1.3–117)	2.2 (0.6–9.8)	68.8 (0.6–532)
Day 1, 6 h	952 (321–1,158)	18.9 (4.0–101)	2.6 (0.8–8.6)	105 (0.6–494)
Day 2	536 (165–2,535)	20.0 (4.2–94.1)	2.7 (1.1–8.6)	98.9 (0.6–461)
Day 3	440 (241–3,955)	17.7 (1.2–99.2)	3.0 (0.9–7.8)	107 (6.7–509)

Analysis of variance	Nucleosomes		CEA		CYFRA 21-1		CA 19-9	
	p value	p adj.	p value	p adj.	p value	p adj.	p value	p adj.
Postoperative vs. preoperative								
Effect of therapy group	<0.001	<0.001	NS		NS		NS	
Effect of days of therapy	NS		NS		NS		NS	
Effect of interaction	NS		NS		NS		NS	
Postoperative vs. relapse								
Effect of therapy group	0.001	0.003	0.012	0.036	0.031	0.093	NS	
Effect of days of therapy	NS		0.008	0.024	NS		0.002	0.006
Effect of interaction	NS		NS		NS		NS	
Preoperative vs. relapse								
Effect of therapy group	NS		NS		NS		NS	
Effect of days of therapy	0.011	0.033	0.001	0.003	NS		NS	
Effect of interaction	NS		NS		NS		NS	

RCT = Radiochemotherapy; adj. = adjusted; NS = not significant.

Discussion

Multimodal therapy strategies have shown to increase the treatment efficacy in many cancer types, such as colorectal cancer. In the preoperative as well as in the postoperative setting, radio- and chemotherapy often are combined because they attack cancer cells at different levels. As the combined application of both modalities is associated with enhanced toxicity, predictive and prognostic markers are required to indicate early whether the treatment applied will be successful or whether it should be changed.

As apoptosis is one of the main mechanisms leading to the demise of cancer cells, blood markers reflecting the apoptotic process and also tumor-associated antigens might be well suited for this purpose. Typical cell death products are circulating nucleosomal DNA fragments which are created after endonucleatic cleavage of the chromatin and subsequent release by dying cells. Recent work has shown that the course of nucleosomes correlated with the response to radiotherapy and chemotherapy in patients with various cancers [12, 20]. Additionally, the initial course of nucleosomes during radiochemotherapy was a significant indicator for the time to progression

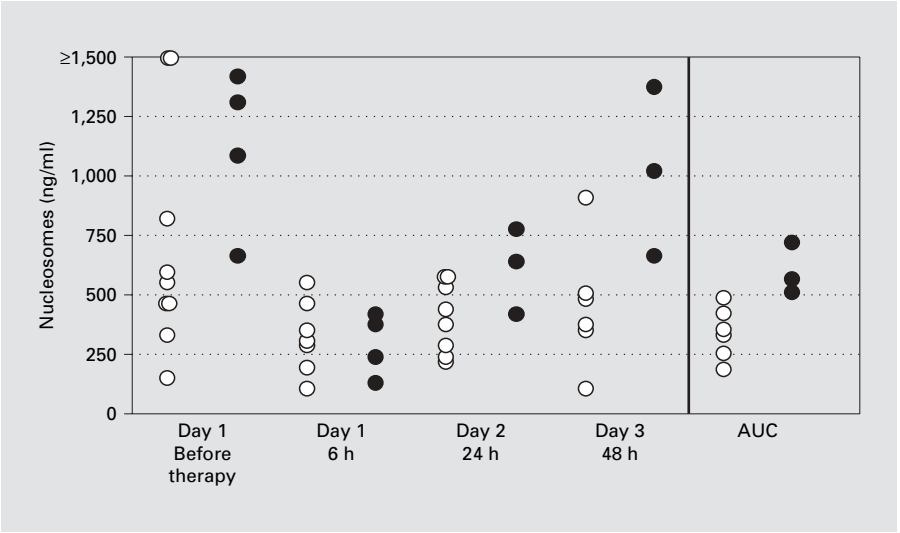

Fig. 2. Distribution of nucleosomes during the first 3 days of treatment in preoperatively treated colorectal cancer patients without progression (○) and with progression (●) of disease. The discriminating power between both groups concerning the prediction of therapy response is p = 0.052 (analysis of variance) for single nucleosome values and p = 0.028 (Wilcoxon text) for AUC.

Table 3. Medians of nucleosomes, CEA, CA 19-9 and CYFRA 21-1 in preoperatively treated patients without (No Prog.) and with progression (Prog.) of disease

	Nucleosomes		CEA		CYFRA 21-1		CA 19-9	
	No Prog.	Prog.	No Prog.	Prog.	No Prog.	Prog.	No Prog.	Prog.
Day 1, 0 h	942	2,084	3.0	9.1	1.4	2.4	11.7	32.0
Day 1, 6 h	126	512	1.9	8.7	1.3	2.7	11.3	30.9
Day 2	208	1,103	2.3	8.3	1.9	2.5	10.6	13.6
Day 3	238	1,775	2.0	10.1	1.7	2.8	7.7	13.9
Analysis of variance: No Prog. vs. Prog.								
Effect of therapy group	p = 0.052		NS		NS		NS	
Effect of days of therapy	p = 0.005		NS		NS		NS	
Effect of interaction	NS		NS		NS		NS	

NS = Not significant.

in patients with pancreatic cancer [21]. Further in patients with advanced lung cancer during chemotherapy, nucleosomes were able to predict the response to therapy early already after the first application [22].

Here we investigated the course of nucleosomes and of other tumor-associated antigens in colorectal cancer patients during the initial phase of radiochemotherapy asking whether they are able to predict the response to therapy early. As radiochemotherapy was applied in some patients preoperatively to reduce tumor volume before resection, in others who suffer from local tumor relapse, and finally in others still after surgical resection of the primary tumor, it was further analyzed whether the quantity of the marker release was associated with the presence of tumor burden during therapy.

Corresponding with earlier findings in pancreatic cancer patients [21], we also observed in most of the patients with colorectal cancer a temporary decrease of the nucleosome concentration 6 h after the first irradiation followed by a rapid increase and a subsequent decline. It is known that already the first irradiation damages DNA effectively by single and double DNA strand breaks, and further by changes of the ionic setting, production of free radicals and other reactive products [23]. As described by Shi-

nomiya [24], high irradiation doses predominately cause irreparable DNA damage and induce early, premitotic apoptosis, whereas low doses cause less severe DNA damage which, in consequence, does not lead to acute cell death. Instead, cells are arrested in their cell cycles to enable the repair of the damaged DNA. In case of insufficient or impaired repair mechanisms, cells will undergo delayed, postmitotic apoptosis [25, 26]. This might explain the temporary decrease immediately after therapeutic irradiation with 1.8 Gy per fraction and the delayed maximum after 1–3 days of the appearance of apoptotic products in circulation, which is in line with earlier in vitro findings, too [27].

The nucleosome values in postoperatively treated patients were significantly lower compared with patients still having tumor masses and receiving preoperative or relapse therapy. During postoperative radiochemotherapy, nucleosome levels increased only slightly after the start of treatment and remained at low levels during the whole follow-up time. On the one hand, this observation was surprising as we expected a considerable number of 'normal cells' being damaged by the therapy applied. On the other hand, it was in concordance with recent in vitro findings which showed a significantly higher release of nucleosomal DNA fragments after irradiation of lung cancer cells than of 'normal' bronchoepithelial cells, particularly at low doses [28].

Concerning the tumor-associated antigens CEA and CYFRA 21-1, of which the latter has also been described as an apoptotic marker [29], the levels during postoperative therapy were notably lower than in the presence of tumor burden, too. Additionally, the concentrations of CEA, CA 19-9 and CYFRA 21-1 were further enhanced in patients with tumor relapse compared to those with primary tumors. As in recurrent disease cancer cells often have spread beyond the organ frontiers via venous, arterial or lymphatic ways, the higher levels of cancer antigens shed from these cells are well comprehensible. Unlike in our present results, nucleosome levels in postoperative and primary-treated pancreatic cancer patients were earlier found to be comparable. However, this was due to an incomplete resection with remaining tumor burden in all pancreatic cancer patients undergoing surgery [21].

The potential for the early prediction of therapy response by apoptotic markers and tumor-associated antigens was investigated in patients who were treated preoperatively to reduce tumor volume before resection. During this time 9 patients had at least stable disease whereas 4 suffered from progressive disease. The concentrations of nucleosomes were lower in patients with 'no progression' than in those with progression. Even if for single days, the difference was only borderline significant ($p = 0.052$), it became more evident when these values were integrated in the AUC ($p = 0.028$). Our observations are consistent with earlier findings in pancreatic cancer patients when a small AUC predicted a longer progression-free interval [21]. A tendency of higher values in the nonresponsive group was also valid for the tumor-associated antigens CEA, CA 19-9 and CYFRA 21-1; however, they could not discriminate between the nonprogressive and progressive group. As CEA and CA 19-9 have a considerably longer half-life in circulation (2–8 days), fast changes of their concentrations in serum were not expected. In contrast, nucleosomal DNA fragments, which after the occurrence of cell death are more quickly released into and eliminated from circulation [13, 30], are supposed to reflect more accurately the quantity of dying cells at a certain time point. Thus, patients with smaller or less aggressive tumors, which are linked with lower cell death rates, less pronounced release of nucleosomal DNA into circulation, and/or more effective systems to eliminate apoptotic products from circulation, seem to be associated with a better outcome after radiochemotherapy.

In conclusion, the course of nucleosomes during the initial phase of radiochemotherapy, reflected by the AUC, has turned out to be a valuable marker for the early estimation of the response to therapy already in this limited set of colorectal cancer patients. Further prospective studies are warranted to validate these findings.

Acknowledgements

The assays for the determination of nucleosomes, CEA, CYFRA 21-1, and CA 19-9, were provided by Roche Diagnostics, Germany.

References

1 American Cancer Society: Cancer Facts and Figures. Washington, American Cancer Society, 2000.

2 Kornberg R, Lorch Y: Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. Cell 1999;98:285–294.

3 Luger K: Structure and dynamic behavior of nucleosomes. Curr Opin Genet Dev 2003;13:127–135.

4 Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S: A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. Nature 1998;391:43–50.

5 Oliveri M, Daga A, Cantoni C, Lunardi C, Millo R, Puccetti A: DNase I mediates internucleosomal DNA degradation in human cells undergoing drug-induced apoptosis. Eur J Immunol 2001;31:743–751.

6 Platt N, da Silva RP, Gordon S: Recognizing death: the phagocytosis of apoptotic cells. Trends Cell Biol 1998;8:365–372.

7 Lichtenstein AV, Melkonyan HS, Tomei LD, Umansky SR: Circulating nucleic acids and apoptosis. Ann NY Acad Sci 2001;945:239–249.

8 Holdenrieder S, Stieber P, Bodenmüller H, Fertig G, Fürst H, Schmeller N, Untch M, Seidel D: Nucleosomes in serum as a marker for cell death. Clin Chem Lab Med 2001;39:596–605.

9 Holdenrieder S, Stieber P, Förg T, Kuehl M, Schulz L, Busch M, Schalhorn A, Seidel D: Apoptosis in serum of patients with solid tumours. Anticancer Res 1999;19:2721–2724.

10 Kuroi K, Tanaka C, Toi M: Clinical significance of plasma nucleosome levels in cancer patients. Int J Oncol 2001;19:2721–2724.

11 Holdenrieder S, Stieber P, Bodenmüller H, Busch M, Von Pawel J, Schalhorn A, Nagel D, Seidel D: Circulating nucleosomes in serum. Ann NY Acad Sci 2001;945:93–102.

12 Holdenrieder S, Stieber P, Bodenmüller H, Busch M, Fertig G, Fürst H, Schalhorn A, Schmeller N, Untch M, Seidel D: Nucleosomes in serum of patients with benign and malignant diseases. Int J Cancer 2001;95:114–120.

13 van Nieuwenhuijze AE, van Lopik T, Smeenk RJ, Aarden LA: Time between onset of apoptosis and release of nucleosomes from apoptotic cells: putative implications for systemic lupus erythematosus. Ann Rheum Dis 2003;62:10–14.

14 Holdenrieder S, Eichhorn P, Beuers U, Samtleben W, Schoenermarck U, Zachoval R, Nagel D, Stieber P: Nucleosomal DNA fragments in autoimmune diseases. Clin Chem 2005;10:14–15.

15 Fishbein TM, Lui J, Wang L, Li Y, Boros P: Increased apoptosis is specific for acute rejection in rat small bowel transplant. J Surg Res 2004;119:51–55.

16 Lo YM, Rainer TH, Chan LY, Hjelm NM, Cocks RA: Plasma DNA as a prognostic marker in trauma patients. Clin Chem 2000;46:319–323.

17 Rainer TH, Wong LK, Lam W, Yuen E, Lam NY, Metreweli C, Lo YM: Prognostic use of circulating plasma nucleic acid concentrations in patients with acute stroke. Clin Chem 2003;49:562–569.

18 Geiger S, Holdenrieder S, Stieber P, Hamann GF, Bruening R, Ma J, Nagel D, Seidel D: Nucleosomes in serum of patients with early cerebral stroke. Cerebrovasc Dis 2006;21:32–37.

19 Atamaniuk J, Vidotto C, Tschan H, Bachl N, Stuhlmeier KM, Muller MM: Increased concentrations of cell-free plasma DNA after exhaustive exercise. Clin Chem 2004;50:1668–1670.

20 Holdenrieder S, Stieber P: Therapy control in oncology by circulating nucleosomes. Ann NY Acad Sci 2004;1022:211–216.

21 Kremer A, Wilkowski R, Holdenrieder S, Nagel D, Stieber P, Seidel D: Nucleosomes in pancreatic cancer patients during radiochemotherapy. Tumor Biol 2005;26:44–49.

22 Holdenrieder S, Stieber P, von Pawel J, Raith H, Nagel D, Feldmann K, Seidel D: Circulating nucleosomes predict the response to chemotherapy in patients with advanced non-small cell lung cancer. Clin Cancer Res 2004;10:5981–5987.

23 McMillan TJ, Tobi S, Mateos S, Lemon C: The use of DNA double-strand break quantification in radiotherapy. Int J Radiat Oncol Biol Phys 2001;49:373–377.

24 Shinomiya N: New concepts in radiation-induced apoptosis: 'premitotic apoptosis' and 'postmitotic apoptosis'. J Cell Mol Med 2001;5:240–253.

25 Sionov RV, Haupt Y: The cellular response to p53: the decision between life and death. Oncogene 1999;18:6145–6157.

26 Verheij M, Bartelink H: Radiation-induced apoptosis. Cell Tissue Res 2000;301:133–142.

27 Mirzaie-Joniani H, Eriksson D, Sheikholvaezin A, Johansson A, Löfroth PO, Johansson L, Stigbrand T: Apoptosis induced by low-dose and low-dose-rate radiation. Cancer 2002;94:1210–1214.

28 Holdenrieder S, Stief J, Bergner A, Gamarra F, Mitlewski A, Nagel D, Huber RM, Stieber P: Nucleosomes indicate the in vitro radiosensitivity of irradiated broncho-epithelial and lung cancer cells. Tumor Biol 2004;25:321–326.

29 Sheard MA, Vojtesek B, Simickova M, Valik D: Release of cytokeratin-18 and -19 fragments (TPS and CYFRA 21-1) into the extracellular space during apoptosis. J Cell Biochem 2002;85:670–677.

30 Gauthier VJ, Tyler LN, Mannik M: Blood clearance kinetics and liver uptake of mononucleosomes in mice. J Immunol 1996;156:1151–1156.