Circulating Nucleosomes and Cytokeratin 19-Fragments in Patients with Colorectal Cancer During Chemotherapy

STEFAN HOLDENRIEDER1, LUBOŠ HOLUBEC JR2, ONDREJ TOPOLCAN2, JINDRICH FINEK2 and PETRA STIEBER1

1Ludwig-Maximilians-University Munich, Klinikum Grosshadern, Munich, Germany;
2Charles University Prague, Medical Faculty Pilsen, Pilsen, Czech Republic

Abstract. Background: Intracellular components, such as nucleosomal DNA and cytokeratins, are released from cells during apoptosis, which occurs spontaneously in elevated amounts in patients with various tumors, as well as during treatment with chemotherapeutic drugs. Patients and Methods: Sera of 30 patients with colorectal cancer, stage Dukes' B to D, who received adjuvant (N=15) or palliative (N=15) chemotherapy, were investigated. The response to therapy was objectified by imaging techniques after 3 cycles of therapy. Circulating nucleosomes and cytokeratin 19-fragments (CYFRA 21-1; both by Elecsys, Roche) were determined before (day 1) and after (day 3) each cycle of chemotherapy. Results: Nucleosomal DNA typically increased during the chemotherapeutic cycles – most notably in the first 2 cycles (median: INC1: 22% and INC2: 49%) – being applied as adjuvant or palliative therapy. Whereas patients with adjuvant therapy mainly had declining baseline values (median: BV1-2: –21%, BV1-3: –36%), those with palliative treatment showed equal or increasing values (median: BV1-2: 30%, BV1-3: 0%). Within the group of patients with palliative treatment, those with progression of disease (N=6) had increasing baseline values (median: BV1-3: 33%) and high increases during the cycles (median: INC1: 232% and INC2: 60%); those with no progression showed decreasing baseline values (median: INC1: –22%) and mainly decreases during the cycles (median: INC1: –38% and INC2: –52%). CYFRA 21-1 showed less change during chemotherapeutic cycles (median: INC1: 17%, INC2: 22%; palliative: INC1: 9%, INC2: 11%) and concerning the baseline values (adjuvant: BV1-2: 1%, BV1-3: –6%; palliative: BV1-2: –3%, BV1-3: 9%). The absolute levels of pretherapeutic values were higher for those with progression (median: 2.1 ng/mL) than for those without progression (1.3 ng/mL). Conclusion: The kinetics of circulating nucleosomes in patients with colorectal cancer show characteristic differences between adjuvant and palliative chemotherapy as well as concerning the response to palliative chemotherapy.

Colorectal cancer is one of the most common malignant diseases in the Western world and has shown a relatively stable incidence rate during recent decades (1, 2). Due to a rising awareness of the disease and due to new programs for the early detection of colorectal cancer that have been initiated in various countries in recent years, the tumor is often detected in its early stages (3-5). In Dukes’ A disease, surgical resection of the neoplasm can lead to complete cure, whereas in Dukes’ B and C stage of rectal cancer, and at least in Dukes’ C stage in colon cancer, adjuvant chemotherapy is applied in many countries (6, 7). Despite the recent efforts to improve the situation, the diagnosis of colorectal cancer is frequently only established when symptoms are obvious and the disease has already spread to various organ sites. In these cases, the only treatment options are palliative chemotherapeutic schedules (6). Along with the development of promising new drugs, there is a growing need to establish prognostic scores, to monitor the therapies effectively and to estimate the usefulness of a specific regimen as early as possible.

For monitoring purposes, the carcinoembryonic antigen (CEA) has been used for years as a reliable and valid biochemical correlative of the tumor response to therapy (8-10). In addition to CEA, CA19-9 has revealed independent prognostic power for colorectal cancer (11, 12). However, the early prediction of therapy response is still an open question that we intended to address with this study. In advanced lung cancer, circulating nucleosomal DNA and
Cytokeratin 19-fragments (CYFRA 21-1) have been reported to indicate early on the efficacy of chemotherapy (13). These intracellular components are released from cells during apoptosis as it occurs spontaneously in malignant tumors, or as it is induced during treatment with chemotherapeutic drugs (14). Despite the different pathologies, we analyzed the kinetics of circulating nucleosomes and CYFRA 21-1 in the sera of patients with colorectal cancer during adjuvant and palliative chemotherapy in order to elucidate their potential for monitoring the response to therapy in this setting.

Patients and Methods

We included 30 patients with colorectal cancer in our study, under the care of the Medical Faculty Pilsen, Czech Republic. The detailed patients’ characteristics are listed in Table I. They received adjuvant (N=15) or palliative (N=15) chemotherapy, consisting of 5-fluorouracil and folinic acid according to the De Gramonte regimen (N=15), and 5-fluorouracil, folinic acid and irinotecan according to the FOLFIRI regimen (N=15). The study was approved by the local ethics committee. Written informed consent was obtained from all patients.

Blood was drawn before (day 1) and after (day 3) the first, second and third cycle of chemotherapy. After centrifugation, the sera were stabilized for nucleosome determinations and stored at -70°C. For the measurement of nucleosomes and CYFRA 21-1, samples were transferred to the University Hospital Munich-Grosshadern, Germany. Nucleosome and CYFRA 21-1 concentrations were quantified by an automatized electrochemoluminescence method (Elecsys, Roche Diagnostics, Penzberg, Germany).

The clinical response to therapy was objectified by imaging techniques, mostly sonography or computed tomography, after 3 cycles of chemotherapy. The outcome was classified according to the World Health Organisation criteria: during palliative treatment, remission was defined as tumor reduction ≥50%, progression as tumor increase ≥25% or manifestation of new lesions, and stable disease as an intermediate status in between these modalities. During adjuvant treatment, progression as the reappearance of new tumor lesions was distinguished from continuing remission.

Differences in nucleosome and CYFRA 21-1 concentrations between patients with adjuvant and palliative therapy, as well as between patients (during palliative treatment) with progression (N=6) and no progression (N=9), were analyzed with regard to the increases of the values within each therapeutic cycle (INC1, INC2, INC3) and the baseline values between various cycles (BV1-2, BV1-3).

Results

In a pilot study, a rapid increase in the concentration of nucleosomes after application of chemotherapy, followed by a decline in the treatment-free interval, were observed in patients with various cancers (15). In patients with progressive disease, the baseline values before each cycle often increased constantly, whereas they decreased in patients with remission of disease (15, 16) (Figure 1). Therefore, in the present study, concerning circulating nucleosomes and

| Table I. Clinical characteristics of the patients. |
|-------------------------------|--------|--------|
| Age                          | 63.5   | 37-76  |
| Gender                       | Number | Percentage |
| Female                       | 11     | (36.7%) |
| Male                         | 19     | (63.3%) |
| Stage                        |        |         |
| Dukes’ B                     | 10     | (33.3%) |
| Dukes’ C                     | 10     | (33.3%) |
| Dukes’ D                     | 10     | (33.3%) |
| Chemotherapy regimen         |        |         |
| De Gramonte                  | 15     | (50.0%) |
| FOLFIRI                      | 15     | (50.0%) |
| Chemotherapy modality        |        |         |
| Adjuvant therapy             | 15     | (50.0%) |
| Palliative therapy           | 15     | (50.0%) |
| Response to adjuvant therapy |        |         |
| Remission                    | 14     | (93.3%) |
| Progression                  | 1      | (6.7%)  |
| Response to palliative therapy |  |         |
| Remission                    | 2      | (13.3%) |
| Stable disease               | 7      | (46.7%) |
| Progression                  | 6      | (40.0%) |

Figure 1. Course of nucleosomes in a patient with colorectal cancer (Dukes’ D) during palliative chemotherapy. Baseline values (---) increased along with progression of the disease.
CYFRA 21-1 in patients with colorectal cancer, the precyclic baseline values and the increases from day 1 to 3 during each cycle, were of particular interest when investigating whether there are differences between adjuvant and palliative therapies and whether these parameters are meaningful for the therapy monitoring or the prediction of therapy response.

In the overall evaluation including all patients receiving adjuvant or palliative therapy, nucleosomes increased in many patients during the chemotherapeutic cycles, most notably during the first 2 cycles (median: INC1: 22%, INC2: 49%, INC3 10%). In contrast, the changes of CYFRA 21-1 during the various cycles were less pronounced (median: INC1: 13%, INC2: 15%, INC3 1%) (Figures 2 and 3).

When comparing patients with adjuvant and palliative therapy, those with postsurgical adjuvant treatment mainly presented declining nucleosome baseline values (medians: BV1-2: –21%, BV1-3: –36%) and almost constant CYFRA 21-1 baseline values (median: BV1-2: +1%, BV1-3: –6%); those with palliative treatment showed equal or increasing nucleosome values (median: BV1-2: 30%, BV1-3: 0%) and only slight changes in CYFRA 21-1 levels (median: BV1-2: –3%, BV1-3: +9%) (Figures 4 and 5).

Concerning the response to therapy in the adjuvant group, only one patient suffered from progressive disease in the staging investigations; we, therefore, omitted the further evaluation of the parameters according to the therapy response. In the palliative group, 2 patients had remission, 7 stable and 6 progressive disease.

Patients with progression of disease during palliative therapy had increasing nucleosome baseline values (median: BV1-3: 33%) and high increases during the cycles (median: INC1: 232% and INC2: 60%); those with no progression showed decreasing baseline values (median: BV1-3: –22%) and mainly decreases during the cycles (medians: INC1: –38% and INC2: –52%) (Figure 6).

With regard to CYFRA 21-1, in patients with progressive diseases during palliative therapy, mainly declining baseline values (median: BV1-3: –32%) were observed, along with slight increases during the cycles (median: INC1: 18% and INC2: 10%), whereas those patients with no progression had slightly increasing baseline values (median: BV1-3: 18%) as well as slight increases during the cycles (median: INC1: 0% and INC2: 11%). However, it should be mentioned that the absolute level of the pretherapeutic CYFRA 21-1 values

![Figure 2. Increases (INC) of nucleosomes during the first 3 cycles of chemotherapy. Medians are indicated by bold lines.](image)
Figure 3. Increases (INC) of CYFRA 21-1 during the first 3 cycles of chemotherapy. Medians are indicated by bold lines.

Figure 4. Kinetics of the baseline values (BV) of nucleosomes during the first 3 cycles of adjuvant and palliative chemotherapy. Medians are indicated by bold lines.
Figure 5. Kinetics of the baseline values (BV) of CYFRA 21-1 during the first 3 cycles of adjuvant and palliative chemotherapy. Medians are indicated by bold lines.

Figure 6. Differences in the kinetics of nucleosomes concerning the response to palliative chemotherapy. The median increases during cycles 1 and 2 (INC) and the median changes in the baseline values (BV cycle 1-3) are indicated for patients with progressive (PROG) and non-progressive disease (NO PROG) during therapy.
Discussion

As the tumor stage at time of diagnosis is crucial for the further prognosis of patients with colorectal cancer, many efforts currently focus on the early detection of these malignant tumors (3-5). However, despite earlier tumor diagnosis and surgical resection, many patients will suffer from later recurrence or systemic spread of the disease and will be treated by surgery or by palliative chemotherapeutic schedules (6). Along with the development of new and effective drugs, there is a growing need to establish methods for therapy monitoring and the early prediction of therapy response that would be helpful for the individual management of disease. For monitoring purposes, carcinoembryonic antigen is a well established biochemical marker (8-10). It is mainly used to complete the staging investigations together with imaging techniques that are performed after several cycles of chemotherapy. However, blood parameters with a shorter half-time would potentially enable an earlier prediction of the therapy response. In a recent study on patients with advanced lung cancer, nucleosomal DNA and cytokeratin 19-fragments indicated the efficacy of chemotherapy even after one treatment cycle (13). Although colorectal cancer constitutes a completely different pathology, the kinetics of these apoptotic markers released after chemotherapy-induced cell death might be meaningful for the prediction of therapy response in this tumor entity, too.

As expected, a substantial increase in the concentrations of nucleosomes during the various treatment cycles, that was irrespective of the adjuvant or palliative therapy modality, was observed in many patients. This might be due to the effective damage of malignant and also of non-malignant cells that both contribute to the release of nucleosomal DNA into the circulation, as is also seen after radiotherapy (17, 18). However, in the follow-up, the presence and extent of tumor masses might be relevant for the spontaneous release of nucleosomal DNA. Concerning the preclinical baseline values, constant or often even increasing levels were observed in patients during palliative treatment protocols. In contrast, baseline values mostly decreased in patients who received adjuvant therapies. This phenomenon can be further explained by the higher percentage of patients with progressive disease in the palliative rather than in the adjuvant treatment group, which has previously shown to be associated with increasing baseline values (15, 16).

Within the group of patients receiving palliative therapies, the difference in the nucleosomal baseline courses could be confirmed according to treatment response: although there was a considerable overlap between both groups at the individual level, patients with progression exhibited, on average, an increase whereas those with no progression showed a decrease from cycle 1 to 3. Additionally, the nucleosome values increased considerably during the first and second cycle of chemotherapy from days 1 to 3 in all but one patient with later progression, whereas they mainly decreased or increased to a lesser extent in patients with stable disease or remission. This observation corresponds with our earlier results in patients with advanced lung cancer receiving chemotherapy (13, 16): those with later progression showed a high peak and an insufficient decrease during the first week of the treatment, while those with good response had a lower peak and a complete decrease. Several factors might play a role in this context: patients with poor response might potentially be suffering from more aggressive tumors that are related to a high spontaneous turnover of cell death and proliferation, and that might consist of a high portion of undifferentiated cells which are prone to be killed by antitumor therapy (16).

In contrast to nucleosomes, cytokeratin 19-fragments were only present in low concentrations in the serum of colorectal cancer patients. In the follow-up, they showed only small alterations during and between the various cycles of chemotherapy. Between the groups of patients with adjuvant and palliative treatment schedules, there were only marginal differences, too. Finally, concerning the response to palliative chemotherapy, we could not observe different CYFRA 21-1 courses in responsive and non-responsive patients. However, the absolute CYFRA 21-1 levels in patients with later progression were slightly higher than in those without progression. These results contradict the prominent role of CYFRA 21-1 for the monitoring and the early prediction of therapy outcome in patients with lung cancer (13). However, also at low levels, CYFRA 21-1 might potentially contain prognostic information in colorectal cancer.

For the early prediction of therapy response, studies will have to include further promising markers. Because chemotherapeutic drugs are known to be effective at distinct cell cycle levels, they may include those that particularly are able to distinguish between premitotic and postmitotic apoptosis (19-21). Furthermore, multiparametric models will have to be established to fully benefit from the predictive information given by all the relevant clinical and biochemical parameters.

Conclusion

Although the number of patients in this study was limited, the kinetics of circulating nucleosomes in patients with colorectal cancer showed characteristic differences between adjuvant and palliative chemotherapy, and also with regard to the response to palliative chemotherapy. Currently, we are
extending our study to show whether nucleosomes and other apoptotic markers will be helpful for the early prediction of response to palliative treatment in colorectal cancer patients.

References