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Calorimetry-based profiling of blood plasma from colorectal cancer patients

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ABSTRACT

Background: Differential scanning calorimetry (DSC), a highly sensitive technique for resolving thermally-induced protein folding/unfolding transitions, recently was recognized as a novel tool for disease diagnosis and monitoring. To further elaborate this approach we have applied DSC in a study of blood plasma from patients with colorectal cancer (CRC) at different stages of tumor development and localization.
Methods: Blood plasma from patients diagnosed with CRC was analyzed by DSC. The CRC thermograms were compared to those of healthy individuals and patients with gastric cancer and non-cancerous soft tissue inflammation. The thermodynamic parameters: excess heat capacity and enthalpy of the transitions corresponding to the most abundant plasma proteins, as well as transition and first moment temperatures were determined from the calorimetric profiles.
Results: The calorimetric profiles of blood plasma from CRC patients are found to be distinct from those of healthy individuals and those of patients with soft tissue, non-cancerous inflammation. Generally the CRC thermograms exhibit reduced heat capacity of the major albumin/globulin-assigned thermal transitions, lower enthalpy and a featureless high temperature region compared to healthy individuals.
Conclusions: A classification of blood plasma proteome from patients with colorectal cancer (CRC1, CRC2 and CRC3 groups, and subgroups within each group $CRC1_{1-2}$, $CRC2_{1-2}$ and $CRC3_{1-2}$) is proposed based on the derived thermodynamic parameters.
General significance: The presented data demonstrate a proof of principle and confirm that the DSC technique has a potential to monitor changes in the CRC blood plasma proteome. This study is a further step toward the validation of calorimetry as a diagnostic tool.

1. Introduction

Differential scanning calorimetry (DSC) appeared as a novel tool in biomedicine in 2007 when Chaires and co-workers revealed its potential for disease diagnostics performed on blood plasma [1–3]. A typical DSC thermogram of blood plasma has been established for healthy individuals, whereas for diseased subjects (including oncopatients) it was shown to differ [1–6].

DSC is a highly sensitive technique that precisely measures the thermally-induced conformational transitions of biomolecules and allows the determination of the thermodynamic parameters of protein denaturation (folding/unfolding) [7]. The interest in applying DSC on blood plasma in cancer diagnostics is high since it is non-invasive for the patients and provides fast in situ monitoring of changes in the thermodynamic behavior of blood plasma/serum. Modifications of plasma DSC thermogram of a diseased subject are thought to be related either to alterations in plasma protein content or to shifts in their characteristic denaturation temperature due to ligand and/or tumor-specific secretory marker binding (interactomics). So far the DSC approach was applied to small cohorts of cancer patients [1–3,5,6]. Recently DSC investigation on multiple myeloma has been published by our research group [8]. These studies have identified specific disease-related calorimetric features however the validation of DSC as a useful tool for disease diagnostics and monitoring requires large-scale investigations of various diseases. As a step toward this goal, hereby, we report on an in-depth DSC study on a heterogeneous cohort of colorectal cancer (CRC) patients diagnosed with different tumor-node-metastasis (TNM) stages.

Although the DSC data reveal heterogeneity in the thermograms some common characteristics for the majority of the CRC plasma

Abbreviations: DSC, differential scanning calorimetry; CRC, colorectal cancer; GC, gastric cancer; STI, non-cancerous soft tissue inflammation; TNM, (tumor, lymph nodes, metastasis) stage; T_{max}, temperature maximum of the transitions; T_{FM}, temperature of the first moment; c_P^{ex}, excess heat capacity; ΔH_{cal}, total enthalpy

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Table 1
Clinical description of the CRC patients.

Number of patients	74
Mean age (interval)	63 ± 11
Gender, M/F	M (39), F (35)
Stages	55 cases
T2-T4N0M0	54.7%
T4-N1-2M0	18.9%
T4N0M1-M2	3.8%
T4N1-N2M1-M2	22.6%
After surgery	19 cases

profiles are to be noted: (i) strong variation in the albumin- and globulin-related thermal transitions and (ii) featureless high temperature region of the DSC thermograms compared to that of healthy individuals. Patients diagnosed with another epithelial cancer, namely gastric cancer (GC), show thermograms similar to certain CRC subtypes. In addition a pilot study on patients with soft tissue inflammation (STI) is also presented, which reveals features distinct from the ones found for CRC patients, GC patients and healthy individuals.

2. Material and methods

2.1. Individuals

The population under study included a control group of 32 healthy volunteers (of age 34–81 years, the mean age and the standard deviation being 57 ± 14), 74 patients with CRC (63 ± 11 years of age) (Table 1), 8 patients with GC (59 ± 15 years of age) and 6 with non-cancerous soft tissue inflammation. The patients selected for the study have not received chemo- and radiotherapy. Among the 74 CRC patients 55 varied in the disease stage determined by the TNM staging system: 54.7% of the CRC patients were without lymph node involvement and distal metastasis (T1-T4N0M0), 18.9% were lymph node positive and without distal metastasis (T4N1-2M0), 3.8% had distal metastasis but were lymph node negative (T4N0M1-2) and 22.6% were lymph node positive and had distal metastasis (T4N1-2M1-2), whereas the other 19 had undergone surgical intervention (Table 1).

2.2. Blood sample preparation

3 mL blood was centrifuged for 15 min at 900 RCF in Venosafe plastic tubes (Plasma gel). The supernatant (blood plasma) was carefully removed, diluted twice in PBS buffer, and used for subsequent DSC measurements.

2.3. Protein analysis

Protein concentration of the plasma samples is determined by the biuret method [9]. CEA, CA19-9 and CA 125 tests and staging determination were performed at the clinical laboratory of the National Oncology Hospital, Sofia, Bulgaria.

2.4. Differential scanning calorimetry

DSC thermograms were detected using DASM1 (Privalov, BioPribor)-build-in highly sensitive calorimeter and the data were analyzed with the Origin software package. The samples were heated at a scanning rate of 0.8 °C/min from 20 °C to 95 °C. The thermograms were normalized to the total protein content and corrected for the instrumental base line. The following parameters were determined: transition temperature, T_{max} (temperature maximum of the successive transitions); temperature

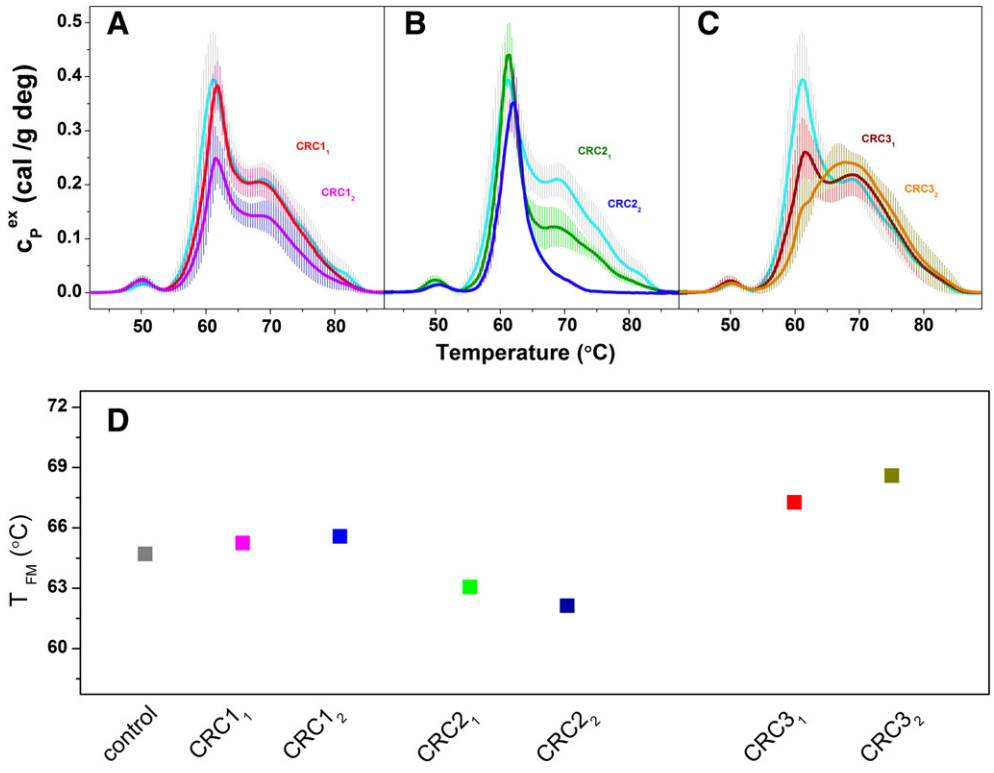

Fig. 1. Characteristic DSC profiles of blood plasma proteome from patients with CRC. Average DSC profiles (solid lines) and standard deviations (shading) are shown for the healthy individuals (cyan line/light gray shading, all panels) and CRC subgroups. Panel A: $CRC1_1$, red solid line and pink shading and $CRC1_2$, magenta solid line and blue shading; panel B: $CRC2_1$, olive solid line and green shading and $CRC2_2$, blue solid line and violet shading; panel C: $CRC3_1$, wine solid line and red shading and $CRC3_2$, orange solid line and dark yellow shading. For clarity the CRC groups are denoted in different colors in panels A–C. The first moment temperatures (T_{FM}) and their standard deviations for each study group are shown on panel D in the same color as the shading of the respective averaged thermograms in panel A–C.