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Differential scanning calorimetry (DSC) analysis of human plasma in melanoma patients with or without regional lymph node metastases

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Abstract Melanoma malignum (MM) is a common type of skin cancer, and its incidence is increasing in the general population. We aimed to detect blood plasma components with differential scanning calorimetry (DSC) in 15 white adult MM patients, who had histopathologically diagnosed, operable cutaneous MM without any distant metastases. We observed that thermal changes (second T_m, calorimetric enthalpy) in blood plasma showed correlation with tumor thickness and the extent of regional invasion. Further studies are needed to elucidate these relationships, but our preliminary work has provided DSC should be a new tool for the early diagnosis and monitoring of MM patients.

Keywords DSC · Melanoma malignum · Lymph node metastases

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Introduction

Cutaneous melanoma is the most malignant tumor of the skin and its incidence is on the rise [1, 2]. There is convincing evidence from epidemiologic studies that endogenous (genetic markers, skin type) and exogenous (ultraviolent irradiation) risk factors are in the development of melanoma malignum (MM) [3]. Early detection of primary melanoma assures increased survival, advanced MM has a poor prognosis and survival. The treatment primarily includes surgical removal of the tumor and adjuvant therapy (chemo-, immuno-, and radiation therapy) [4]. The clinicopathological stage of the melanoma patients can determine by pathological evaluation of the primary lesion and of the dissected lymph nodes, as well as by routine examinations (lactate dehydrogenase test, chest X-ray, ultrasound of the abdominal cavity, and computed tomography (CT) or positron emission tomography combined with CT) [5]. In 1969, Clark et al. proposed staging criteria for lesions on the basis of skin invasion levels [6]. Subsequently, Breslow evidenced the importance of the primary melanoma thickness in millimeters, and this index became one of the most important prognostic indicators, in association with data on ulceration, mitosis, and regression [7]. Moreover, regional lymph node (Sentinel) status has emerged as an accurate method for evaluating the draining lymph node basin, allowing for the generation of valuable prognostic information. Sentinel lymph node biopsy became a compulsory phase for patients with tumor thickness >1 mm [8, 9]. Beside routine clinical follow-up with the unaided eye additional techniques are being used to follow these high risk patients sequentially. Recently, there is a need for more studies and methods to monitor in MM patients in any stages.

Differential scanning calorimetry (DSC) is unsurpassed for understanding the stability of biological systems. DSC directly measures the stability and unfolding of a protein, lipid, or nucleic acid that occur in bio-molecules during controlled increase or decrease in temperature, making it possible to study materials in their native state. The DSC thermogram is a unique signature for bio-molecules reflecting the normal or pathomorphological changes under given solution conditions. Therefore, DSC technique allows demonstrating the thermal consequences of conformation changes in different bio-molecules not only in the animal experiences, but in several surgical and oncological clinical studies [10–14].

Aim of the study

Current research focused on developing the application of DSC approach as a new diagnostic and monitoring method for MM patients with or without regional lymph node metastases.

Clinical

Patients and methods

Patient selection

Fifteen white adults (12 men and 3 women; median age 58.6 years) had been operated in the Department of Dermatology, Venereology and Oncodermatology of University Pécs. According to general checkup of patients, patients had operable cutaneous MM without any distant metastases. From routine histopathological parameters, tumor thickness was evaluated according to Breslow which parameter changed from 0.5 to 8.5 mm in our patients. The most striking invasion values were in the Clark level II and IV in this study. Regional lymphatic infiltration was evaluated as a prognostic factor, and the sentinel lymph node was positive in seven cases, wherein adjuvant therapies (Interferon alpha-2b, Telecobalt) were started. The protocol was approved by regional ethical committee of Pécs University (27.06.2008/3220).

Blood sample preparation

Preoperatively peripheral blood samples were collected from the patients ($n = 15$) and from healthy controls ($n = 5$). Blood samples were collected into the Vacutainer tubes containing EDTA (1.5 mg/mL of blood) centrifuged at 1600g for 15 min at 4 °C to separate plasma fraction from cell components. Native plasmas were stored at −80 °C until DSC measurement.

DSC measurements

The thermal unfolding of the human plasma components was monitored by SETARAM Micro DSCII calorimeter. All experiments were conducted between 0 and 100 °C. The heating rate was 0.3 K/min in all cases. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μL sample volume in average. Reference sample was contained normal saline (0.9% NaCl). The sample and reference samples were equilibrated with a precision of ±0.1 mg. There was no need to do any correction from the point of view of heat capacity between sample and reference samples. The repeated scan of denatured sample was used as baseline reference, which was subtracted from the original DSC curve. Calorimetric enthalpy was calculated from the area under the heat absorption curve using two-point setting SETARAM peak integration.

Results and discussion

Malignant melanoma is one of the most aggressive malignancies in human and is responsible for almost 60% of lethal skin tumors. Its incidence has been increasing in white population in the past two decades. The disease was classified by Clark and coworkers into intraepidermal (Clark level I), invades papillary dermis (Clark level II), fills papillary dermis (Clark level III), invades reticular dermis (Clark level IV), and invades subcutaneous fat (Clark level V) [15]. Clark's level is a related staging system, used in conjunction with Breslow's depth, which describes the level of anatomical invasion of the melanoma in the skin. Clark's level has prognostic significance only in patients with very thin (Breslow's depth <1 mm) melanomas.

Adequate resection of the specimens and sentinel lymph node biopsies are important factors in management of MM. However, there is no definite proof that longevity of patients is affected by routine laboratory tests [16]. The human plasma proteome holds great promise as a convenient specimen for disease diagnosis and therapeutic monitoring. Moreover, blood samples may be easily obtained from patients by minimally invasive, safe procedure. The novel calorimetric assays are described that provide a new window to view the properties of the human plasma proteome [7, 11, 17, 18].

This study investigated the thermal changes of human blood plasma components in melanoma patients with or without regional lymph node metastases by DSC. Overview of 15 patients' thermograms, we observed their individual characteristics compared to healthy controls. Similar observations have been described by Garbett et al.

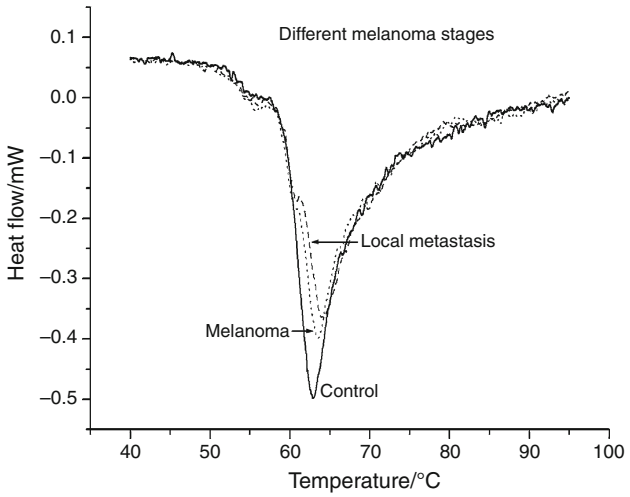

Fig. 1 DSC scans of human plasma in healthy controls, in MM patients with or without local metastases. Downward deflection represents endotherm process. *Solid line* control, *dotted line* MM without local metastasis, *dashed line* MM with local metastasis

in their important calorimetric studies, where demonstrated average thermograms for individuals diagnosed with various diseases (Lyme disease, rheumatoid arthritis) and cancers (endometrial, ovarian, lung), among others in five patients with MM. These data suggest that each type of cancer or disease may have a characteristic signature in their thermogram [19].

In the present study, our results showed that comparison of DSC scan of healthy controls with the curves of cases with melanoma and local metastases (Fig. 1), the DSC measurements showed at least two marked different thermal domains during the denaturation. Examination of DSC data in different clinical stages of MM patients should observed correlation with melanoma thickness and the extent of regional invasion. The first T_m was slightly influenced by the Breslow's depth and the Clark level (I and III) (see Tables 1, 2), but it can be seen a difference in the melting enthalpies (see Fig. 1). The second T_m's and the calorimetric enthalpy changes demonstrated a significant difference of the melanoma depth dependence in 1.1–8.5 mm range and in Clark levels of II–IV (see Fig. 1 as well as Tables 1, 2). These thermal parameters have been changed significantly in comparing with the control samples which were: T_m's 56 and 63 °C, $\Delta H \sim 1.5$ J/g. In the pathologic samples and in the progress of the decease, one can separate a third thermal component between the first and second T_m (see Fig. 1). It is at around 62 °C, and it is shifted to higher temperature in case of local metastasis. The surprising jump out of all thermal data in 1.1–2.0 mm Breslow's depth range as well as the opposite change in the tendency of T_{m2} and ΔH need further investigation with increased number of patients and with finer filtering.

Table 1 DSC data of human blood plasma in healthy controls and in MM patients according to Breslow's depth

Human blood plasma	T_{m1}/°C (Mean)	T_{m2}/°C (Mean)	ΔH/J g^{-1} (Mean)
Healthy controls ($n = 5$)	56	63.3	1.53
Breslow's depth (mm)			
0.5–1.0 ($n = 2$)	55.8	64.1	1.13
1.1–1.5 ($n = 2$)	56.3	63.5	1.61
1.6–2.0 ($n = 3$)	55.75	67.15	1.3
2.1–3.0 ($n = 3$)	56.16	62.18	1.45
3.1–4.0 ($n = 2$)	55.8	63.2	1.37
4.1–6.0 ($n = 2$)	55.7	63.9	1.25
6.1–8.5 ($n = 1$)	55.8	64.5	1.23

T_{m1} and T_{m2} melting temperatures, ΔH calorimetric enthalpy change of endotherm process

Table 2 DSC data of human blood plasma in MM patients according to Clark level of melanoma

Clark level	T_{m1}/°C (Mean)	T_{m2}/°C (Mean)	ΔH/J g^{-1} (Mean)
I ($n = 2$)	55.2	64.6	1.14
II ($n = 3$)	55.8	63	1.45
III ($n = 5$)	55.7	64.63	1.286
IV ($n = 5$)	56.05	60.4	1.43

T_{m1} and T_{m2} melting temperatures, ΔH calorimetric enthalpy change of endotherm process

The same could be the conclusion in case of Clark levels II and IV.

Moreover, patients' thermograms are shifted toward higher denaturation temperatures. These changes were confirmed in the literature, where the average thermogram was obtained from duplicate DSC runs on samples from 100 healthy individuals and from five MM patients' sample also. In this study, the disease thermograms are apparently localized in a higher temperature range. These unique appearances present a key utility of this technology as a diagnostic method [20].

These facts are important for many reasons: DSC measurement is suitable not only to clear skin cancer diagnosis, but also to separate the different stages of MM patients and to monitor the actual stage of individual's disease. However, there are no data in the literature indicating the possible diagnostic and staging method of human blood plasma by DSC in MM patients. Similar findings have been described in another report, where applied the DSC method to investigate its utility for disease staging. Gynecologic oncology samples analyzed by the method yielded progressively shifted thermograms charting the advance of cervical cancer from pre-invasive cervical lesions through each stage of invasive carcinoma. The distinction between

normal and high-grade squamous intraepithelial lesions is significant and indicates the utility of the DSC method for the rapid screening of cervical cancer [20]. The exact explanations of these results are not yet known. However, DSC analysis of plasma from diseased individuals revealed significant changes in the thermogram which are suggested to result not from changes in the concentration of the major plasma proteins but from interactions of small molecules or peptides with these proteins [20, 21].

In summary, this is the first report examined thermal changes by DSC on human blood plasma in MM patients with different clinical stages. Blood collection is a simple procedure and convenient to perform, and the DSC thermogram confirmed unique signature for human plasma components reflecting the normal, the pathomorphological changes and staging differences in melanoma patients. Further studies are needed to elucidate these relationships, but this preliminary study indicates great potential for the application of DSC as a clinical diagnostic tool, for example, during disease grading and staging processes.

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