Serum total gangliosides level: clinical prognostic implication

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Abstract

Purpose: The gangliosides overexpression contributes to the development of skin melanoma. The purpose of this study was to determine if the total gangliosides serum levels might predict the tumor growth in patients with melanoma or if the transfer of shed cell gangliosides reflects the implication in the clinical prognostic of these patients. Patients and Methods: Total gangliosides serum levels were measured in the cryopreserved serum by estimating lipid-associated sialic acid in 761 patients before surgical resection of melanoma, in 406 patients with precancerous pigmentary lesions, and in 410 healthy individuals. This study was performed at the Dermatovenereological Research Center, Bucharest, Romania, during 1991–2010. All sera obtained after surgical resection of melanocytic tumors were analyzed to see if adjuvant therapy (chemo-, immuno-, immunochemo-therapy) induced gangliosides changes in melanoma patients and if the responses were correlated with survival. Results: Total gangliosides serum levels were higher in melanoma patients than in precancerous melanocytic lesions patients or in healthy individuals. Larger tumors in Breslow index and more advanced stage of disease were correlated with higher total gangliosides serum values. Augmented total gangliosides serum levels after melanoma adjuvant treatment were predictive for decreased overall survival, whereas decreased total gangliosides serum levels were predictable for improved overall survival. Conclusions: A marker for early melanoma complications and survival may be the total gangliosides serum level.

Keywords: melanoma, gangliosides, survival.

Introduction

The incidence and mortality rate of cutaneous melanoma have been increasing more rapidly over the last decades. Several studies have been published on the role of glycosylation and gangliosides (sialic acid containing glycosphingolipids) in the development and therapy of those tumors [1–3].

Glycosylation modulates several cellular functions such as: proliferation, cell–cell and cell–matrix adhesion, signal transduction, apoptosis, angiogenesis, the expression of oncogenes and antioncogenes, growth factor receptors [4–6]. Tumor progression is associated with overexpression of gangliosides. Furthermore, metastatic melanoma shed these molecules into their microenvironment [7–9] and much suggests that soluble gangliosides contribute to tumor-induced immunosuppression [7–11].

Exogenous gangliosides may regulate melanoma growth by modulating the signaling pathways, including those related to 3',5'-adenosine cyclic monophosphate (cAMP) [12]. The melanoma cells constitutively release chemotactants and other active mediators to stimulate migration and activation of macrophages, monocytes, granulocytes, keratinocytes, fibroblasts, platelets as well as other cellular components of innate immunity [13].

The gangliosides promote tumor metastases and angiogenesis by modulating the autocrine growth factor production [6, 12, 13] and protect the tumor against the host immune system [1, 2, 6, 11].

In this study, it was explored the possible significance of gangliosides in the oncogenic transformation and tumor progression of melanoma in conjunction with clinicopathological features. In fact, nowadays, “polarization” of gangliosides as targets for active immunotherapy in cancer, came from the recent work reported in 2009 by the National Cancer Institute pilot project for prioritization of cancer antigens [1, 14].

Patients and Methods

Patient’s selection

The present study was performed in healthy individuals without clinical evidence of cancer (n=410), in patients with precancerous or dysplastic lesions of melanocytes (n=406) and patients with melanoma (n=761). The patients were attended at the Dermatovenereological Research Center in Bucharest, Romania, from 1991 until 2010.

Patients were monitored for:
- histological and immunohistological criteria [15] of primary tumors;
- the status of seric gangliosides levels in relation
with: sex, age, site of primary tumor, histological type, Clark level, Breslow index, sites of metastases, different therapeutic strategies;
• relapse-free interval and survival time for determining oncospecific treatment effects.

All samples were obtained with the permission of the individuals before their inclusion in the study, after informed patients consent according to the Declaration of Helsinki.

Isolation and characterization of gangliosides

The isolation procedures for gangliosides were described previously by Ariga T, in 1991. In brief, the total lipids were extracted from serum with chloroform/methanol (2:1, 1:1, v/v) and chloroform/methanol/water (30:60:8) successively. The combined extracts were then applied to a DEAE-Sephadex A-25 column and further eluted with chloroform/methanol/0.8 M sodium acetate (30:60:8). The acidic lipid fraction was further eluted with chloroform/methanol/0.8 M sodium acetate (30:60:8) successively. The combined extracts were evaporated to dryness, and the residue was redissolved in chloroform/methanol/water and then desalted by Sephadex column chromatography. Gangliosides was assayed by resorcinol-HCl [16, 17]. Protein bound sialic acid represents the difference between total sialic acid, lipid bound sialic acid and free sialic acid.

Statistical analysis

The chi-square test was used to test for differences in distribution of total gangliosides serum levels between groups; and the Student’s t-test was used to mean differences for a comparison between the groups. P-values less than 0.05 are considered as statistically significant. All data were evaluated using EpiInfo 2008 version.

Results

The main demographical, clinical features and histopathological findings (site of primary tumor, histological type, Clark levels, sites of metastases, systemic therapy) of melanoma patients in the study are shown in Table 1.

| Table 1 – Characteristics of melanoma patients, precancerous pigmentary lesions patients and healthy individuals |
|--------------------------------------------------|----------------|----------------|----------------|
| Characteristics                                | Melanoma patients | Precancerous lesions | Control         |
| Sex                                             | male/female       | 299/462          | 147/259         | 160/250          |
| Age [years]                                     | median/range      | 53 (28–86)       | 49 (22–80)      | 54 (22–86)       |
| Site of primary tumor                           | head-neck/trunk/arm/leg/unknown | 97/402/83/143/36 | – | – |
| Histological type                               | SSM/NM/ALM/MLM/UM/unknown | 318/213/88/43/17/72 | – | – |
| Clark level                                     | I/II/III/IV/V/undetermined | 0/161/201/259/97/43 | – | – |
| Metastases                                      | skin/liver/lung/SNC/bone/comboination/undetectable | 111/76/133/42/6/60/353 | – | – |
| Treatment                                       | chemotherapy/immunotherapy/immunocemotherapy/without treatment | 49/218/104/37 | – | – |

SSM – superficial spreading melanoma; NM – nodular melanoma; ALM – acral lentiginous melanoma; MLM – melanoma of malignant lentigo; UM – unclassified melanoma.

There were observed no significant statistical differences between gangliosides serum level and sex, age, site of primary tumors, histological type, sites of metastases. The 761-melanoma patients were between 28 and 86-year-old. The parameters count: white blood cell over 3500/mm³, platelets over 100 000/mm³, hemoglobin concentration over 10 g/dL and serum biochemistry and urine analysis were normal.

Histochemical and immunohistochemical techniques were used for the diagnosis of melanoma. The battery of antibodies for the melanoma diagnosis is composed from HMB45, Melan A, S100, vimentin and cytokeratin HMB45 is positive in most of the studied melanomas (Figure 1). Melan A is a monoclonal antibody that is positive in most of melanomas. The label is usually cytoplasmatic (Figure 2) S100 marks the majority of the melanocytic lesions. The label is nuclear and cytoplasmatic (Figure 3), vimentin is present both in nevus cells and in melanoma cells; their label is cytoplasmatic (Figure 4). Cytokeratin is negative melanoma cells (Figure 5). Total gangliosides levels were measured in the cryopreserved sera by estimating lipid associated sialic acids from 406 patients with precancerous pigmentary lesions (18.86±8.27 mg/dL) vs. 410 healthy individuals (18.02±2.78 mg/dL). There were observed no significant statistical differences between those study groups (p>0.05). When compared with healthy subjects (18.02±2.78 mg/dL serum), patients with melanoma had higher concentrations of gangliosides (62.87±30.84 mg/dL serum) (p<0.005) (Table 2).

Serum total gangliosides levels were significantly higher in patients with advanced melanoma vs. melanoma patients without metastatic nodes (80.14±19.26 mg/dL vs. 44.29±17.61 mg/dL, p<0.05).

The transition of melanoma from radial growth phase to vertical growth phase was accompanied by several molecular changes leading to uncontrolled increased levels of gangliosides in circulation. There were registered a medium increase of 16.8%. Moreover, the ratio between lipid bound sialic acid and proteins bound sialic acid in patients with melanoma vs. other clinical situations studied.
Table 2 – Serum total gangliosides levels from melanoma patients before surgical resection vs. precancerous pigmentary lesions patients and healthy individuals

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of patients</th>
<th>Total serum gangliosides [mg/dL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy (control)</td>
<td>410</td>
<td>18.02±2.78</td>
</tr>
<tr>
<td>Precancerous pigmentary lesions</td>
<td>406</td>
<td>18.86±8.27</td>
</tr>
<tr>
<td>Melanoma patients:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Melanoma patients without metastatic nodes</td>
<td>353</td>
<td>44.29±17.61</td>
</tr>
<tr>
<td>• Advanced melanoma</td>
<td>408</td>
<td>80.14±19.26</td>
</tr>
</tbody>
</table>

To notice that the gangliosides serum levels is importantly modified after surgical removal of the tumor. In Table 3, we presented the average of seric gangliosides for every Breslow index before and after surgical removement.

Table 3 – Total gangliosides serum levels from melanoma patients before and after surgical resection vs. Breslow index

<table>
<thead>
<tr>
<th>Breslow index [mm]</th>
<th>No. of patients</th>
<th>Total serum gangliosides before surgical resection [mg/dL]</th>
<th>Total serum gangliosides after surgical resection [mg/dL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.75</td>
<td>142</td>
<td>32.67±9.23</td>
<td>28.69±11.17</td>
</tr>
<tr>
<td>0.75–1.5</td>
<td>180</td>
<td>50.88±19.74</td>
<td>36.37±11.60</td>
</tr>
<tr>
<td>1.5–3</td>
<td>201</td>
<td>73.12±23.19</td>
<td>57.82±21.63</td>
</tr>
<tr>
<td>&gt;3</td>
<td>118</td>
<td>89.15±22.31</td>
<td>60.35±28.11</td>
</tr>
<tr>
<td>Undetermined</td>
<td>120</td>
<td>76.19±24.11</td>
<td>56.10±19.14</td>
</tr>
</tbody>
</table>

Figure 1 – Cutaneous melanoma; HMB45 positive in tumoral cells, ob. 10×.

Figure 2 – Cutaneous melanoma; Melan A positive in tumoral cells, ob. 20×.

Figure 3 – Cutaneous melanoma; S100 positive in tumoral cells, ob. 20×.

Figure 4 – Cutaneous melanoma; vimentin positive in tumoral cells, ob. 20×.

Figure 5 – Cutaneous melanoma; cytokeratin 34 negative in tumoral cells, ob. 20×.

Figure 6 – The glycoconjugate serum levels correlated with melanoma transition from radial to vertical growth.
\section*{Discussion}

Melanomas of the skin overexpress a variety of gangliosides. Gangliosides are strongly expressed in metastatic melanoma cells and less frequently expressed in nevus cells. The gangliosides are released into the tumor microenvironment and circulation, where they suppress a variety of cells-mediated immune functions [1]. This discovery suggests that gangliosides may be a valuable target for diagnostic, prognostic and therapeutic exploration.

In the current report, we confirmed that aberrant and elevated gangliosides levels represent potential marker for malignancy. Serum levels of gangliosides in healthy individuals and patients with precancerous pigmented lesions were insignificantly modified (Table 2). However, gangliosides serum levels do not increased significantly in patients with early-melanoma. Elevated gangliosides levels had been observed in advanced melanoma patients and had been shown to be an important marker of tumor progression. In addition, experimental results might indicate the association of serum total gangliosides levels with transition of melanoma from radial growth phase to vertical growth phase, with thickness of primary tumor. Many studies indicate that aberrant glycosylation is a result of initial oncogenic transformation, as well as a key event in induction of invasion and metastases [5, 6]. The concept of glycosylation-dependent promotion or inhibition of tumor progression has developed in conjunction with clinicopathological features [1, 5]. The gangliosides serum concentration has a significant impact on survival. Low values of gangliosides in patients without metastatic nodes and without tumoral ulceration suppress tumoral progression leading to higher postoperative survival rates [18]. High values of gangliosides in patients with nodal metastases and primary tumor ulceration promote invasion and metastases leading to the decrease of the relapse-free interval and low survival rates of patients.

The important alteration in the levels of gangliosides in short-term may be useful as marker in the detection of early melanoma complications. Serum gangliosides decrease after surgical treatment is correlated with improved overall survival and relapse-free survival. Increased levels of gangliosides after surgical remove are associated with metastasizing process. Overexpression of gangliosides in melanoma cells with little or no expression in normal adult tissues represents a target for antimitastatic reagents. In malignant melanoma, the authors identified the following gangliosides: GM2, GM3, GD2, GD3, GM1, GD1a, GD1b, GT1b, GQ1a, O-AcetylgD3. Targeting gangliosides antigens is a matter of concern due to the possibility of inducing autoimmune responses [19]. The criteria for prioritization [14, 20] of cancer antigens in vaccine development include:

- therapeutic function;
- immunogenicity;
- role of antigen in oncogenicity;
- specificity;
- expression level and percent of antigen-positive cells;
- stem cell expression;
- number of patients with antigen-positive cancer;
- number of antigen epitopes;
- cellular location in antigen expression.

\section*{Conclusions}

Total gangliosides serum level may be a useful marker in the detection of early melanoma complications. Gangliosides serum level predicts survival and may be of therapeutic and prognostic value in the management of melanoma.

\section*{References}


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