Gastric cancer increases transmigratory potential of peripheral blood monocytes by upregulation of β1- and β2-integrins

Andrzej Eljaszewicz¹, Michal Jankowski¹,³, Małgorzata Wiese-Szadkowska⁴, Lidia Gackowska⁴, Jacek Michalkiewicz⁴, Wojciech Zegarski²,³, Marcin Moniuszko¹

¹Department of Regenerative Medicine and Immune Regulation, Medical University of Bialystok, Poland
²Department of Surgical Oncology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University of Torun, Poland
³Oncology Centre – Prof Franciszek Lukaszczyk Memorial Hospital, Bydgoszcz, Poland
⁴Department of Immunology, Faculty of Pharmacy, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Poland

Introduction

Macrophages represent predominant component of leukocyte infiltrate in many tumors, including gastric cancer [1, 2]. Due to their pleiotropic biological activities they act as orchestrators of immune response within tumor. They may play pivotal role in tumor development acting as tumor suppressors (M1 phenotype – classically activated cells) or tumor supporters (M2 phenotype – alternatively activated cells) [1, 3]. Unfortunately, majority of macrophages within tumor microenvironment acquire the latter phenotype and are referred to as tumor associated macrophages (TAMs). Macrophage polarization depends on the immune modulatory properties of tumor and stroma cells, that can interact locally within tumor tissue or affect peripheral precursors (monocytes) [4].

Recruitment of monocytes into tumor microenvironment is a hallmark of cancer development and progression [5, 6]. Notably, migration of peripheral blood cells to the side of tumor growth is controlled by different soluble factors, namely cytokines, chemokines, growth factors and metabolites [7, 8]. On the other hand, vascular and epithelial junctions represent a barrier for leukocyte migration [9]. Interestingly, monocyte transmigration through vessel wall is possible due to the presence of membrane adhesion molecules, including proteins belonging to β1 and β2-integrin family [10]. Leukocyte adhesion molecules interact with their ligands expressed on cytokine activated endothelium and allows monocytes to avoid forces exerted by the rapid blood flow in vasculature. Consequently, monocytes start to roll along apical endothelial surface until complete immobilization and transmigration [11]. Unfortunately, to date the molecular, humoral and cellular mechanisms that control monocyte trafficking in cancer are not fully elucidated. Therefore, here we aimed to evaluate whether systemic activation of peripheral blood monocytes observed in gastric cancer patients increases transmigratory potential of these cells.

Material and methods

Patients

15 normal donors and 40 gastric cancer patients, successively qualified to stomach resection at Chair of Surgical Oncology, Prof. F. Lukaszczyk Memo-
rial Centre of Oncology in Bydgoszcz (Poland), were enrolled to the study (Table 1). None of the patients received chemotherapy and radiotherapy before or was subjected to surgery or blood transfusion for at least six month before blood acquisition. Furthermore, none of the patients showed any clinical or cellular sings on ongoing infection. Peripheral blood was collected upon the approval of the Bioethical Committee of the Collegium Medicum in Bydgoszcz. Each participant was familiarized with the objectives of the study and expressed written consent.

Flow cytometry

100 μl of fresh heparin-anticoagulated blood was stained with panel of mouse anti-human monoclonal antibodies (Table 2). Stain-then-lyse protocol was used as previously described [12]. Appropriate fluorescence-minus-one (FMO) and isotype controls were used for every staining for setting compensation and to assure correct gating. Samples were analyzed by using FACScan flow cytometer (Becton Dickinson) and at least 40 000 events were collected. Next, collected data were analyzed by using FlowJo version 7.6.1. (TreeStar). Used gating strategy is presented on Fig. 1.

Statistics

Statistical analysis was carried out using GraphPad Prism 6 software (GraphPad Software). U Mann-Whitney test was used. The differences were considered statistically significant at \( p < 0.05 \). The results are presented as median (interquartile range).

Table 1. Clinical characteristics of study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gastric cancer</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>40</td>
<td>17</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>62.79 (30-86)</td>
<td>58.35 (49-68)</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>13/27</td>
<td>8/9</td>
</tr>
<tr>
<td>Stage according to AJCC (frequencies of all)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>12 (30%)</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td>2 (5%)</td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td>14 (35%)</td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>12 (30%)</td>
<td></td>
</tr>
</tbody>
</table>

AJCC – American Joint Committee on Cancer

Table 2. Characteristics of monoclonal antibodies used in the study

<table>
<thead>
<tr>
<th>Name</th>
<th>Clone</th>
<th>Isotype</th>
<th>Format</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14</td>
<td>MoP9</td>
<td>IgG2b</td>
<td>PerCP</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>CD49d (VLA-4 ( \alpha ) subunit)</td>
<td>9F10</td>
<td>IgG1</td>
<td>FITC</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>CD49f (VLA-6 ( \alpha ) subunit)</td>
<td>1.BB.460</td>
<td>IgG2b</td>
<td>FITC</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>CD11a</td>
<td>G43-25B</td>
<td>IgG2a</td>
<td>FITC</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>CD11b</td>
<td>1.BB.189</td>
<td>IgG1</td>
<td>FITC</td>
<td>Santa Cruz Biotechnology</td>
</tr>
<tr>
<td>CD11c</td>
<td>B-Ly6</td>
<td>IgG1</td>
<td>PE</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>CD18</td>
<td>6.7</td>
<td>IgG1</td>
<td>PE</td>
<td>Becton Dickinson</td>
</tr>
</tbody>
</table>

PerCP – peridinin chlorophyll protein complex; FITC – fluorescein isothiocyanate; PE – phycoerythrin

Results

Due to the constitutive expression of analyzed \( \beta_2 \)-integrins on the surface of monocytes we analyzed their expression levels. First we found significant increase of CD11a and CD11b expression in gastric cancer patients when compared to normal donors. Additionally, we found no differences in CD11c and CD18 expression level. Next, we found that gastric cancer increase frequencies of CD49d (\( \alpha_4 \) subunit of VLA-4 integrin) and CD49f (\( \alpha_6 \) subunit of VLA-6 integrin) expressing monocytes. Interestingly, we did not observed any differences in expression level of above mentioned molecules.

Discussion

Integrins are membrane glycoproteins controlling numerous physiological processes, including cell adhesion, chemotaxis, and phagocytosis [13]. \( \beta_2 \)-integrins are heterodimeric receptor proteins consisting of \( \alpha \) and \( \beta \) subunits linked by sulphide bridges. All receptors shear common \( \beta_2 \)-chain (CD18) and differ in \( \alpha \) chain variants, namely \( \alpha_L – CD11a; \alpha_M – CD11b; \alpha_X – CD11c \). They are involved in direct adhesion of leukocytes to endothelial cells [14, 15]. Similarly, \( \beta_1 \)-integrins shear common \( \beta_1 \) chain (CD29) and differ in \( \alpha \) subunits. To date, at least 10 different \( \alpha \) chains were discovered including CD49d and CD49f expressed on monocytes. In contrast to \( \beta_2 \)-integrins, the latter represent a group of protein receptor responsible for cell interactions with extracellular matrix [13]. Here, we found that gastric cancer increase frequencies of both VLA-4 and VLA-6 expressing monocytes. Interestingly, Jin et al. showed that VLA-4 is playing leading role in monocyte transmigration to tumor microenvironment [16]. However, in some contrast to previous studies by Zhang et al., they found that this process occurred in the \( \alpha_M \beta_2 \) (CD11b/CD18) integrin independent manner [16, 17]. It seems, that \( \beta_2 \)-integrins may play supportive role in monocyte transmigration process and observed upregulation of CD11a and CD11b expression is a consequence of monocyte activation and their inflammatory phenotype [18]. Notably, in our previous report we found that gastric cancer patients showed increased frequencies of inflammatory (activated) monocytes, namely intermediate (CD14++CD16+) and non-classical (CD14+CD16++) cells [12]. Interestingly, VLA-4 support not only transmigration of inflammatory monocytes but also...
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...the transition of macrophages from classically activated (M1) to pro-tumoral (M2) cells, by Rac2 activation [19]. Therefore, increased frequencies of VLA-4 expressing monocytes may be a consequence of their non-classical activation. Furthermore, VLA-6 was shown to support tumor growth and angiogenesis by promoting tumor infiltration of Tie-2 expressing monocytes and macrophages (TEMs) [20]. In summary, we showed here that peripheral blood monocytes from gastric cancer patients possess high transmigratory potential and are sensitive for non-classical polarization.

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References

Fig. 1. Gating strategy for monocytes. Representative FACS plots demonstrating gating strategy to evaluate CD49d (VLA-4 α subunit), CD49f, CD11a, CD11b, CD11c and CD18 expressing monocytes and fluorescence intensity of analyzed receptors
Fig. 2. β2-integrin expression in peripheral blood monocytes. Summary of analyses of (A) CD11a, (B) CD11b, (C) CD11c and (D) CD18 expression on CD14+ cells. Data are presented as median and interquartile range.

Fig. 3. VLA-4 and VLA-6 expression in peripheral blood monocytes. Summary of analyses of (A) VLA-4 and (B) VLA-6 expressing monocytes and (C) VLA-4 and (D) VLA-6 expression on CD14+ cells. Data are presented as median and interquartile range.
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Address for correspondence
Andrzej Eljaszewicz
Department of Regenerative Medicine and Immune Regulation
Medical University of Bialystok
Waszyngtona 13
15-269 Bialystok, Poland
e-mail: andrzej.eljaszewicz@umb.edu.pl

Marcin Moniuszko
Department of Regenerative Medicine and Immune Regulation
Medical University of Bialystok
Waszyngtona 13
15-269 Bialystok, Poland
e-mail: Marcin.Moniuszko@umb.edu.pl