Potential Stem Cell Marker CD44 is Constitutively Expressed in Permanent Cell Lines of Head and Neck Cancer

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Abstract. Background: Despite significant advances in the use of diagnosis and therapy to treat head and neck squamous cell carcinoma (HNSCC), the prognosis has improved only marginally in the last decades. Thus, there is an enormous need for better understanding of tumor biology and reversely novel immunotherapeutic approaches. It is becoming increasingly obvious that stem cells play an important role in tumor development and progression. The identity of these cells and the underlying cellular and molecular mechanisms are mostly unknown in HNSCC to date. Materials and Methods: Solid HNSCC tumors, as well as permanent HNSCC cell lines, were analyzed by flow cytometry concerning the expression of different putative stem cell marker proteins. Results: Distinct populations of CD44 expressing potential stem cells could be identified in solid tumors of HNSCC patients with strong individual deviations. Surprisingly, the potential stem cell marker CD44 was found to be constitutively expressed on the surface of all the permanent HNSCC cell lines analyzed. Conclusion: CD44+ ‘tumor stem cells’ may play a key role in the establishment of permanent HNSCC cell lines, selecting especially robust cell entities that might drive the progression and metastasis of HNSCC. Individual analysis of ‘tumor stem cell’ markers will be an important tool for innovative therapies and for determining the prognosis of patients with HNSCC.

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common tumor occurring almost exclusively among middle-aged tobacco and alcohol abusers (1). Despite recent advances in the treatment of HNSCC, the clinical outcome is still unsatisfactory (2). The cells of head and neck cancer are known to escape from antitumor immune responses, but the molecular mechanisms responsible for tumor aggressiveness and its response to chemo- and radiation therapies remain mostly unknown (3-7).

In HNSCC it has been shown that CD34 natural suppressor cells in the bone marrow can be mobilized by tumor released granulocyte macrophage colony-stimulating factor (GM-CSF) and that vascular endothelial growth factor (VEGF) subsequently triggers their chemotaxis into the tumor (8, 9). These CD34+ progenitor cells participate in the biogenesis of the HNSCC microenvironment by the formation of a new endothelial cell/vessel system and they are able to restrict the function of HNSCC infiltrating T-cells (10, 11). Their suppressive character has been suggested to act through the production of nitric oxide (NO), as well as transforming growth factor (TGF)-β (12, 13). In addition to these CD34+ progenitor cells it is becoming increasingly evident that different stem cells play an important role in tumor development and progression.

Many questions concerning tumor metastasis and recurrence, as well as the development of chemo- and radioresistance may be answered by the identification and characterization of cancer stem cells which are presumed to possess tumor initiating potential combined with the capacity of self-renewal and multilineage differentiation (14, 15).

Though there is evidence that the majority of carcinomas are clones of one cell, the critical problem in cancer research is still the identification of the responsible cell types (16). Recently, a subpopulation of cells with cancer stem cell properties has been identified in HNSCC. These cells were found to express CD44 and their tumorigenic potential was shown in an immunodeficient mouse model (17).

The identity of additional potential tumor stem cells in HNSCC and their underlying cellular and molecular mechanisms are still mostly unclear to date.

In this work we analyzed the expression profiles of CD44 in different solid HNSCC, as well as various permanent HNSCC cell lines were analyzed using flow cytometry.
Materials and Methods

Cell culture. The permanent HNSCC cell lines BHY (Deutsche Sammlung für Mikroorganismen und Zellkulturen, DSMZ, Germany; (18)), PCI-1, PCI-13 (hypopharyngeal cancer, Pittsburgh Cancer Institute, PA, USA) and FaDu (DSMZ, hypopharyngeal cancer (19)) were analyzed. In addition two pairs of permanent cell lines generated from a tumor and a corresponding metastasis from a laryngeal carcinoma (UT-SCCR 42 A/B) and an oropharyngeal carcinoma (UT-SCC 60 A/B) were analyzed (obtained from the University of Turku, Finland; (20)). Additionally a human breast adenocarcinoma cell line MCF7 (21) and an ovarian cell line BG-1 (22) were analyzed. The cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Gibco, NY, USA) supplemented with 10% FCS, 1 mM glutamine, and 0.1 mM sodium pyruvate. All the compounds were purchased endotoxin tested.

Human tissue samples. After written informed consent was provided the tumor tissue specimens were obtained during the standard surgical procedure. Simultaneously tumor draining lymph nodes were obtained by neck dissection and examined by a pathologist. The tissue specimens were transported in sterile saline and processed immediately after excision. The use of human tissues for research purposes was approved by the Ethics Committee of the University of Lübeck.

Preparation of single-cell suspensions. The HNSCC specimens were washed several times and carefully minced into small pieces in sterile serum free RPMI medium (RPMI 1640 supplemented with 100 units/ml penicillin, 1 mM glutamine, and 100 units/ml streptomycin). The tumor tissue was digested with collagenase type VIII (1.5 mg/ml, Sigma, Munich, Germany) and DNase type I (1.0 µg/ml) for 120 min at 37°C with gentle agitation. The resulting cell suspensions were washed in PBS, resuspended in PBS containing trypsin/EDTA, and filtered through a 40-µm nylon cell strainer (Falcon; Becton Dickinson Labware, Heidelberg, Germany) into cold RPMI medium containing 10% FCS. Single cell suspensions were used for the flow cytometric analysis.

Flow cytometry. Surface antigen staining was performed as described previously (23) using anti-CD44-PE (Phycoerythrin) conjugated antibodies (Becton Dickinson, Heidelberg, Germany) by incubation on ice for 15 min followed by washing with PBS.

The samples were analyzed on a FACSCanto (Becton Dickinson). Propidium-iodide staining was used to determine the number of dead cells. Data acquisition and analysis were performed using the FACS DIVA software (Becton Dickinson).

Results

Individual expression profiles of CD44+ in solid HNSCC. The presence of distinct CD44 expressing subpopulations in HNSCC was confirmed and strongly deviating frequencies in the individual tumors were demonstrated (Figure 1).

Additional flow cytometric analysis demonstrated that these CD44+ subpopulations were negative for lineage markers (CD3, CD16, CD19, CD20 and CD56).

Discussion

In this work the presence of CD44 expressing cell populations in head and neck squamous cell carcinoma which were identified as strongly differing in individual population size was demonstrated. The analysis of various permanent HNSCC cell lines indicated the constitutive expression of CD44 in all the investigated cells. These data strongly suggest the potential role of CD44 positive cells in the establishment of permanent cell lines and thus in HNSCC metastasis formation.

It has been demonstrated in colon cancer that CD44 is involved in antiapoptotic processes, as well as in the regulation of tumor cell migration (24). It is becoming increasingly clear that stem cells are the source of at least some, and perhaps all, kinds of carcinomas. The concept of ‘tumor stem cells’ proposes that a small population of aberrant stem cells are responsible for the maintenance of the malignant tissue of every tumor (25). This concept could explain why tumors often regenerate after being almost completely destroyed by different kinds of treatment. The suggestion that

Table I. Summary of analyzed tumor tissues. Sample numbers, gender (f: female; m: male) and age (in years) of the patients and the tumor entities are shown.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gender</th>
<th>Age</th>
<th>Tumor entity</th>
</tr>
</thead>
<tbody>
<tr>
<td>T448</td>
<td>m</td>
<td>69</td>
<td>laryngeal tumor</td>
</tr>
<tr>
<td>T453</td>
<td>m</td>
<td>45</td>
<td>thyroid gland tumor</td>
</tr>
<tr>
<td>T461</td>
<td>m</td>
<td>56</td>
<td>oropharyngeal tumor</td>
</tr>
<tr>
<td>T466</td>
<td>m</td>
<td>69</td>
<td>laryngeal tumor</td>
</tr>
<tr>
<td>T470</td>
<td>m</td>
<td>68</td>
<td>laryngeal tumor</td>
</tr>
<tr>
<td>T480</td>
<td>f</td>
<td>58</td>
<td>pleomorphic adenoma</td>
</tr>
<tr>
<td>T487</td>
<td>m</td>
<td>68</td>
<td>hypopharyngeal tumor</td>
</tr>
<tr>
<td>T494</td>
<td>f</td>
<td>66</td>
<td>laryngeal tumor</td>
</tr>
<tr>
<td>T495</td>
<td>m</td>
<td>68</td>
<td>hypopharyngeal tumor</td>
</tr>
<tr>
<td>T502</td>
<td>m</td>
<td>65</td>
<td>oropharyngeal tumor</td>
</tr>
</tbody>
</table>

Sample T480 was from a pleomorphic adenoma which is a tumor of the salivary glands, also known as a benign mixed tumor. Rarely, a malignant tumor may arise within this tumor, a phenomenon known as carcinoma ex pleomorphic adenoma. Table I gives an overview of the analyzed tumor samples.

Permanent HNSCC cell lines and CD44 expression. All the analyzed permanent cell lines revealed strong constitutive expression of CD44 on the cell surface (Figure 1). Surprisingly, two analyzed permanent cell lines from gynaecological carcinoma, human breast adenocarcinoma cell line MCF7 and ovarian cell line BG-1, revealed no expression of CD44.
cancer cells possess the same properties as stem cells has been proposed for many years. In 1997 cancer stem cells were identified for the first time in certain types of leukemia (26). The major problem in finding cancer stem cells in different solid tumors was the lack of suitable marker proteins, but in 2003 cancer stem cells were identified in breast tumors (27) when it was shown that the majority of cells in a human breast tumor were incapable of further growth and that only a small population of cells were able to seed new carcinomas (27). Even in 1960 it had been shown that hundreds of thousands of cancer cells need to be injected in order to induce a tumor in an animal model (28).

The origin of cancer stem cells is not yet fully understood. It may be that stem cells themselves suffer different kinds of mutations that result in a loss of control of their self-renewal capacity, which represents the key property of stem cells. While stem cells responsible for maintaining body tissues are able to presumably able to regulate their population size, cancer stem cells have lost this control mechanism (25).

Current concepts of tumorigenesis suggest that cells become malignant only in response to a series of genetic mutations. But this concept does not explain why cells with a life-span of only a few weeks, such as skin cells, accumulate enough mutations to evolve a malignant phenotype (29). The present data corroborate the existence and key role of cancer stem cells which should be the focus of future diagnosis and treatment of human carcinomas.

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References


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