The characterization of PDGFR-alpha and PDGFR-beta expression in malignant non-Hodgkin lymphoma

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Abstract
PDGF receptors play an important role in tumor progression as being part of a group of receptors that are expressed along the membrane of tumor cells. The aim of this study was to evaluate the PDGF receptor expression in follicular and diffuse forms of non-Hodgkin malignant lymphoma. We evaluated 38 biopsy fragments from patients diagnosed with malignant non-Hodgkin lymphoma. We noticed the distribution of PDGFR-alpha and -beta in tumor cells and the immunoreactivity was quantified as the percentage of positive tumor cells. We noticed the presence of score 3, more than 30% of tumor cells positive, for PDGFR-alpha and PDGFR-beta in 50% and 75% cases of follicular non-Hodgkin lymphoma. For diffuse malignant non-Hodgkin lymphomas, score 3 was noted in 23.52% of cases for PDGFR-alpha and 35.29% of cases for PDGFR-beta. This may represent an important therapeutic target in patients who do not respond to conventional therapy, but further research is needed for a careful evaluation of benefits and side effects of PDGFR inhibitors.

Keywords: PDGF receptors, non-Hodgkin lymphomas, angiogenesis.

Introduction
In addition to VEGF, other growth factors also have been shown to have proangiogenic action, such as bFGF, TGF and platelet-derived growth factor (PDGF) [1]. Although the molecular mechanisms of angiogenesis have been largely clarified, successive interference and relationship between these factors during the angiogenic cascade are less understood. This could partly explain the controversial results of antiangiogenic therapy in human cancers.

Although characterized several years ago, PDGF has been studied in relation to tumor angiogenesis only in the past few years. PDGF binds to a large spectrum of mesoderm-derived cells such as fibroblasts, pericytes, glial or mesangial cells [2]. PDGF isoforms bind two distinct receptors, PDGF-alpha and -beta, and this mechanism induces receptor autophosphorylation and activation of several signaling molecules [3].

The activation of the receptors induces cellular response, respectively proliferation and migration. PDGF/PDGFR plays a crucial role in normal development of many organs and in particular, PDGF-B and PDGFR-β are essential for the development of the cardiovascular system. All members of PDGF family have strong angiogenic activity in vivo. The individual chains of PDGF have different affinity for the two receptors. PDGFR-α has high affinity for PDGF-A, -B and -C, while PDGFR-β has high affinity for PDGF-B and -D, these features being demonstrated in vitro [4].

PDGF receptors are expressed in tumor cells and stromal cells in both normal and tumoral tissue. They play an important role in tumor progression as being part of a group of receptors that are expressed along the membrane of tumor cells.

PDGF are expressed in 50–70% of ovarian tumors and are activated in tumor cells through autocrine and paracrine mechanisms [5]. In addition to tumor cells, PDGF are expressed by fibroblasts, endothelial cells, pericytes and tumor stroma [6].

PDGFR-β is usually expressed by the endothelial cells and in several human tumors, capillaries and are often surrounded by perivascular PDGFR-β positive cells. While PDGFR-β expression is common for pericytes associated to tumors, its expression in tumor-associated endothelial cells is apparently lower.

PDGFR-β is expressed in breast cancer and tumor-associated endothelial cells in 69.7% of cases [7]. It has been demonstrated that breast cancer cells induce expression of PDGFR-β but not of PDGFR-α in adjacent endothelial cells. In breast cancer PDGFR-α and PDGFR-β are expressed in the blood vessel wall. A different expression was found in the stromal cells. PDGFR-β is expressed in a significant higher number in...
stromal cells associated to tumor than PDGFR-α. PDGFR are expressed by tumor cells, but with different pattern of expression. PDGFR-α is expressed in less than one third of cases, with moderate intensity and heterogeneous distribution compare with PDGFR-β which was expressed with strong intensity, homogeneous distribution and presence in more than two third of cases [8].

Similar data were found in prostate cancer, where PDGFR expression was associated with tumor progression and PDGFR overexpression has been shown in the majority of bone metastases [9]. These data support the introduction of antiangiogenic anti PDGFR-β therapy in cancers with PDGFR-β positive EC.

Until now, few data in the literature reported the expression of PDGF and of its specific receptors in normal lymph nodes or in patients with malignant lymphoma. Passam FH et al. (2009) [10] demonstrated that in Hodgkin lymphoma neoplastic cell population is expressing VEGF in 48% of cases, HIF-1 alpha in 54% of cases and PDGFR-α in 95% of cases.

The purpose of this study was to evaluate the PDGF receptors expression in tumor cells of follicular and diffuse forms of non-Hodgkin malignant lymphoma.

Materials and Methods

We evaluated 38 biopsy fragments from patients diagnosed with malignant non-Hodgkin lymphoma. The specimens were fixed in 10% buffered formalin for 48 hours, paraffin embedded and serial sections were made for each case. Deparaffinization was followed by rehydration sections with decreasing concentrations of alcohol (100%, 95%, 80%, 70% – for five minutes each). Heat-induced epitope retrieval was performed in citrate buffer pH 6 (Target Retrieval Solution, Dako Glostrup, Denmark) for 20 minutes. Inhibition of endogenous peroxidase with 3% hydrogen peroxide was followed by incubation with primary antibodies (rabbit polyclonal anti-PDGFR-alpha, ready to use and anti-PDGFR-beta, rabbit polyclonal, 1:200, Labvision/Neomarkers, Fremont, CA, USA) for 30 minutes. We used ADVANCE/HRP (Dako Glostrup, Denmark) working system, applied for one hour, followed by 3,3’-diaminobenzidine as chromogen. Nuclei were stained with Lillie’s modified Hematoxylin (Dako Glostrup, Denmark). The entire immunohistochemical procedure was performed using Dako Autostainer Plus (DakoCytonomation).

We evaluated the distribution of PDGFR-α and -β in tumor cells. The immunoreactivity was quantified as the percentage of positive cells according to the following score: 0 for 0% positive cells, 1 if <10% of cells were positive, 2 for 20–30% positive cells, and 3 if the percentage of positive cells for each PDGF receptor was higher than 30%. Microscopic evaluation was performed with Nikon Eclipse E600 microscope and images were acquired using LUCIA G system.

Results

Histopathological evaluation based on routine Hematoxylin and Eosin method revealed four cases of follicular non-Hodgkin lymphoma and 34 types of diffuse malignant non-Hodgkin lymphoma.

In follicular type lymphoma, we noticed the predominant expression of the values 3 and 2, according to our score, compared with values 0 and 1.

The evaluation of PDGFR-α for follicular lymphoma indicated a value 3 of score in two from four cases (50%).

Score 2 was found in one case (25%) (Figure 1a). We have not noticed score 1 in any of the cases and score 0 was present in one case (25%).

The predominant value of the score, which we noticed in the evaluation of PDGFR-β expression in the follicular type of lymphoma was +3. We found more than 30% PDGFR-β positive tumor cells in three from four cases of follicular lymphoma (75%) (Figure 1b). The expression of PDGFR-β but not of PDGFR-α in endothelial cell of adjacent blood vessels was noticed also.

The value 2 of score was found in one case (25%). There were no score 0 and 1 for this category. The expression pattern for PDGFR-β was granular cytoplasmic (Figure 1c).

The immunoexpression for PDGFR-α and -β in follicular non-Hodgkin lymphoma can be summarized in Table 1.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Score 3</th>
<th>Score 2</th>
<th>Score 1</th>
<th>Score 0</th>
</tr>
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<tbody>
<tr>
<td>PDGFR-α</td>
<td>2 cases</td>
<td>1 case</td>
<td>–</td>
<td>1 case</td>
</tr>
<tr>
<td>PDGFR-β</td>
<td>3 cases</td>
<td>1 case</td>
<td>–</td>
<td>–</td>
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</table>

In diffuse forms of non-Hodgkin lymphomas, we observed a relative homogeneous distribution, with similar proportions between the three scores for PDGFR-α. We found followed values: eight of the 34 (23.52%) cases were evaluated with score 3, 10 (29.41%) cases with score 2, eight (23.52%) cases with score 1 and eight (23.52%) cases with score 0. Regarding the distribution pattern of PDGFR-α in diffuse forms of non-Hodgkin lymphomas, we noticed two types: homogeneous in entire tumor area and heterogeneous, with distribution in the tumor cells from the periphery of tumor predominately.

The immunohistochemical reaction for PDGFR-β in diffuse type of non-Hodgkin lymphoma showed positivity in 12 from the 34 cases (35.29%) and in 30% of tumor cells we noticed score 3 (Figure 2a).

The value +2 according to our score was observed in 11 cases (32.35%). The value 1, <10% positive cells was noticed in two cases (5.88%) (Figure 2b). The value 0 of score was found in nine cases (26.47%).

In diffuse non-Hodgkin lymphoma, the expression of PDGFR-α and PDGFR-β can be summarized in the following table (Table 2).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Score 3</th>
<th>Score 2</th>
<th>Score 1</th>
<th>Score 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGFR-α</td>
<td>8</td>
<td>10</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>PDGFR-β</td>
<td>12</td>
<td>11</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>
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![Image of PDGFR-alpha and PDGFR-beta expression](image)

**Figure 1** – Follicular non-Hodgkin lymphoma:
(a) PDGFR-α immunexpression, score 2, ob. ×40;
(b) PDGFR-β immunexpression, score 3, ob. ×40;
(c) PDGFR-β immunexpression, granular cytoplasmic pattern, ob. ×40.

**Figure 2** – Diffuse non-Hodgkin lymphoma, PDGFR-β immunexpression: (a) Score 3, ob. ×20; (b) Score 1, ob. ×20.

### Discussion

The last decade research showed that PDGF and PDGFR are involved in the development and progression of human cancer by autocrine stimulation of tumor cell growth. In addition, PDGF signaling stimulates stromal cells, the best example in this matter being tumor angiogenesis. PDGF and PDGFR are involved in tumor progression by mutations, which increase the expression levels, but also retain the structure and function of these proteins (Betsholtz C, 2004) [4]. The way of action of PDGF/PDGFR axis is predominantly autocrine, unlike of the effects in normal tissues, which has paracrine preference.

Data in literature concerning the expression of these factors in non-Hodgkin lymphoma are insufficient. Our study showed the expression of PDGFR-α in more than 30% of tumor cells in 73.52% of non-Hodgkin lymphoma cases included in this study.

PDGF plays at least three roles that can cause tumor development: autocrine stimulation of tumor cells, angiogenesis stimulation and interstitial tumor pressure control. Blocking autocrine stimulation by inhibiting PDGFR in cell lines in experimental models induced positive results in dermatofibrosarcoma, prostate cancer, ovarian cancer and glioma [11]. PDGF-A produced by tumor cells has been shown to be a major chemotactic factor in stromal cells and the disruption of paracrine signaling of PDGFR-α reduced tumor growth by inhibiting tumor cells and angiogenesis [12].
The expression of PDGF receptor was demonstrated in a wide range spectrum of human tumors, but not in malignant lymphoma. By immunohistochemistry, PDGF was detected in fibrosarcoma, renal cell carcinoma, malignant fibrous histiocytoma and B3 thymoma. Its regular expression is cytoplasmic, with granular pattern and attached to tumor cells, is expressed by small blood vessels and by isolated cells from stroma. So far, there were found no convincing correlations between PDGF expression in tumor cells and the tumor grade, except from glioma.

A greater number of patients with B-cell chronic lymphocytic leukemia than those with NHL had PDGF-B and PDGFR-β expression. At the same time, the PDGF-A expression is present at all stages of B-cell differentiation and suggests its important role B-cell differentiation and proliferation. Expression of PDGF-B and PDGFR-β suggest that the PDGF autocrine signal may be important in malignant transformation in B-cell chronic lymphocytic leukemia [13].

These data showing that VEGF could bind and activate the PDGF-α and PDGF-β receptors expand the repertoire of ligands that could stimulate individually the potential PDGF receptors. However, this raises a crucial issue related to how many different receptors that stimulate the same tyrosine kinase receptor, can induce a different biological response. VEGF expression is very low in normal adult tissues, but the expression of VEGF and PDGF are both regulated significantly during vascular repair phase and in regeneration situations.

Similarly, while the surface profile of PDGF receptor of mesenchymal stem cells can adapt in response to factors in the tumor microenvironment, abundant PDGFR-α receptors and a high rate of PDGFR-α, PDGFR-β appears to be a feature of undifferentiated mesenchymal stem cells. Further studies are needed to completely understand how PDGF receptors are specific ligand. Apparently, PDGFR is not modified only by binding ligands, but is also influenced by the cellular microenvironment [14].

Tumor vessels derive morphological and biochemical from normal vessels. Most antiangiogenic approaches focus on endothelial cell but the assessment of pericytes can provide further details. Abramsson A et al. (2003) [15] demonstrated that pericytes have important functions in tumor vascularization and PDGFR-β is important in recruiting of pericytes. For these reasons, they have indicated PDGFR-β and PDGF-B as promising therapeutic targets. Our study showed a positivity rate of over 30% of tumor cells in 75% of follicular type non-Hodgkin lymphoma and 35.29% from the cases of diffuse type NHL.

The heterogeneity of angiogenesis in human tumors and the different phenotype of EC in different organs suggest that further investigation is needed to understand the interaction between tumor cells and EC in malignancies of various organs. Numerous evidences suggest the role of PDGFR antagonists in the treatment of cancer, especially in tumors in which PDGFR autocrine stimulation is important.

The association of PDGFR antagonists with other therapies such as VEGF inhibitors and chemotherapy seem to have a promising future, but further research is needed for a careful evaluation of benefits and side effects of PDGFR inhibitors.

**Conclusions**

Fifty and more than fifty percent from the patients with follicular non-Hodgkin lymphoma included in our study presented the value +3, for PDGFR-α and PDGFR-β according to our score. In diffuse type of non-Hodgkin lymphoma, we found more than 30% tumor cells positives for PDGFR-α and PDGFR-β in almost similar percent. Our observations regarding PDGFR in lymphomas may represent an important therapeutic target in patients who do not respond to conventional therapy.

**References**

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