Aberrant expression of platelet-derived growth factor (PDGF) and PDGF receptor-α is associated with advanced bone marrow fibrosis in idiopathic myelofibrosis

The expression of members of the platelet-derived growth factor (PDGF) system in bone marrow cells derived from Idiopathic myelofibrosis (IMF) has been investigated by real-time RT-PCR. Increased expression of PDGFs and the corresponding PDGF receptor α could be demonstrated to be a feature of advanced fibrosis in IMF that is not demonstrable in the pre-fibrotic phase of the disease.

The platelet-derived growth factor (PDGF) system of PDGF ligands and receptors is thought to play an important role in the fibrogenic process in idiopathic myelofibrosis (IMF). We quantitatively analyzed PDGF isomers -A, -B, and -C and PDGF receptor α in pre-fibrotic, cellular IMF, advanced IMF with myelofibrosis, and non-neoplastic hematopoiesis. The 3 PDGF isomers and PDGF receptor α were significantly overexpressed in advanced IMF with myelofibrosis. We conclude that overexpression of the PDGF system is a pathogenetic feature of advanced myelofibrosis in IMF.

According to the WHO classification, idiopathic myelofibrosis (IMF) refers to a group of chronic myeloproliferative disorders with currently unknown underlying pathogenesis. It is generally accepted that over time pre-fibrotic, cellular IMF progresses to an advanced stage and bone marrow fibrosis develops. It is also accepted that the proliferation of fibroblasts in bone marrow fibrosis is a reactive rather than a clonal process. The platelet-derived growth factor (PDGF) system of ligands and receptors is widely expressed by a variety of cells and tissues in both physiological and pathological conditions. Among its diverse functions PDGF is known to mediate strong mitogenic signals via PDGF receptors on fibroblasts, endothelial cells, and vascular smooth muscle cells. The analysis of PDGF gene expression by bone marrow cells in patients with severe myelofibrosis has so far often been hampered by the inability to collect these cells (dry tap). This is a plausible reason for why previous expression studies investigated the PDGF system mainly in peripheral cells. In such studies the levels of PDGF in platelets and plasma derived from IMF patients were found to be elevated. Given that the rate of progression and interval to myelofibrosis are very variable in IMF, enhanced PDGF expression could identify cases with an increased risk of progression from the cellular to the fibrotic phase of IMF. On the other hand, PDGF could also be substantially involved in the sustenance of myelofibrosis. In order to investigate potentially aberrant

### Table 1. The gene expression level of PDGF isoforms -A, -B, -C, and PDGF-Rα in cellular IMF, advanced IMF, and in the control group are illustrated as the median followed by the range (in parentheses).

<table>
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<tr>
<th></th>
<th>Cellular IMF</th>
<th>Advanced IMF</th>
<th>Control</th>
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<tbody>
<tr>
<td>PDGF-A</td>
<td>1.1 (0.3-2.6)</td>
<td>2.5 (0.4-8.3)</td>
<td>0.8 (0.2-1.8)</td>
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<tr>
<td>PDGF-B</td>
<td>1.4 (0.2-5.0)</td>
<td>4.4 (1.4-15.8)</td>
<td>1.1 (0.1-3.6)</td>
</tr>
<tr>
<td>PDGF-C</td>
<td>1.2 (0.4-3.2)</td>
<td>1.5 (0.4-10.1)</td>
<td>0.9 (0.4-1.6)</td>
</tr>
<tr>
<td>PDGF-Rα</td>
<td>1.2 (0.4-3.0)</td>
<td>5.7 (0.4-32.0)</td>
<td>1.1 (0.2-2.2)</td>
</tr>
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PDGF expression in IMF subtypes bone marrow trephines from patients with prefibrotic, cellular IMF (n=28) and advanced myelofibrosis (n=29) were retrieved from the archive and classified according to the degree of myelofibrosis essentially as described by Buhr et al. The control group (n=26) comprised cases displaying reactive hyperplasia of megakaryocytopoiesis without any evidence of a neoplastic proliferation.

RNA was extracted from total bone marrow cells and real-time reverse-transcription polymerase chain reaction assays were performed essentially as described elsewhere. Cases of advanced IMF displayed significant overexpression of the PDGF isomers as well as of the PDGF-R α as compared to the expression in cases of cellular IMF and in the control group. Unexpectedly, expression in cases of cellular IMF did not differ substantially from that in non-neoplastic hematopoiesis (Figure 1). The overexpression in advanced IMF was prominent for PDGF-B and PDGF-Rα, with an up to 15-fold (p=0.001) and 32-fold (p<0.0005) increase, respectively. Since fibroblasts in advanced IMF could be a considerable source for PDGF-Rα labeling we applied immunohistochemistry with a monoclonal antibody (MAB322, R&D systems, Minneapolis, USA) in order to delineate cellular origin.

While considerable numbers of megakaryocytes in both cellular IMF and advanced IMF displayed positive cytoplasmic labeling no other cell types, and in particular no stromal cells or endothelial cells, showed demonstrable PDGF receptor α labeling. Megakaryocytes in non-neoplastic hematopoiesis were constantly negative for PDGF-Rα staining (data not shown). There was considerable heterogeneity of labeling intensities for PDGF-B in cellular IMF, advanced IMF, and non-neoplastic hematopoiesis. PDGF-B immunolabeling was not a very sensitive marker to indicate increased fibrosis because a considerable number of cases of advanced IMF with manifest fibrosis stained negative (data not shown). Since the immunohistochemical approach failed to reveal fibroblasts as a relevant source of PDGF-Rα in advanced IMF, other PDGF-R subtypes (such as the β-type) might be involved in the activation of fibroblasts in IMF. Besides complex cellular interactions between the neoplastic clone (i.e. megakaryocytes) and the stroma in IMF, megakaryocytic PDGF-Rα labeling in IMF strongly suggests that PDGF have a role apart from that in the purely fibrogenic process, e.g. involvement in autocrine activation. We conclude that increased expression of PDGFs in advanced IMF reflects disease progression and discriminates the cellular from the fibrotic stage of IMF.

References

2. Heldin CH, Westmark B. Mechanism of action and in vivo role of platelet-derived growth factor. Physiol Rev 1999;79:

Malignant Lymphomas

Is bone marrow trephine biopsy always mandatory in staging Hodgkin’s disease?

We reviewed data from 690 adult patients with Hodgkin’s disease (HD) to determine whether bone marrow biopsy (BMB) is mandatory for all patients. The data suggest that it is not necessary in clinical stage I-IIA. However, bilateral BMTB is recommended in the presence of B symptoms also in patients with localized stage disease.

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The recommended staging procedures for patients with HD include BMTB. The value of this procedure is, however, controversial. The Ann Arbor conference recommendations for staging required BMTB to be carried out in the presence of peripheral blood cytopenia, when bone marrow involvement is doubtful and in clinical stages III and IV.1 The Cotswolds conference recommended BMTB for patients with clinical stages II-IV.2 Literature data report that about 4-15% of patients with HD have bone marrow involvement (BMI); however, the incidence of BMI in patients clinically staged as I-IIA has been shown to be <1%.3,4 Many studies have shown that BMI is not, by itself, an adverse prognostic factor and does not define a specific high risk group requiring a different therapeutic approach.5

We retrospectively examined data from 690 adult patients (over 20 years) treated at our institution between 1993 and 2003, with the aim of evaluating whether BMTB is mandatory for all patients with HD. All patients were submitted to standard staging procedures, including bilateral BMTB. One hundred and fifty patients (22%) were defined as having initial stage disease, 373 (54%) as having intermediate stage disease and 167 (24%) as having advanced stage disease. Initial stage included patients staged I-IIA without risk factors (bulky mediastinal mass, extranodal involvement, massive splenic involvement,