Original Article
The involvement of Galectins in the modulation of the JAK/STAT pathway in myeloproliferative neoplasia

Suzanne M Koopmans1, Freek J Bote1,4, Harry C Schouten2, Jannie Janssen3, Arienne MW van Marion1,5

1Department of Pathology of the Maastricht University Medical Centre, Maastricht, The Netherlands; 2Department of Internal Medicine division of Haematology of the Maastricht University Medical Centre, Maastricht, The Netherlands; 3Department of Clinical Genetics of the University Hospital Maastricht, Maastricht, The Netherlands; 4Department of Pathology of the Haga hospital, The Hague, The Netherlands; 5Department of Pathology of the VieCuri Medical Centre, Venlo, The Netherlands

Received April 16, 2012; accepted May 21, 2012; Epub May 25, 2012; Published June 15, 2012

Abstract: Background: In patients with myeloproliferative neoplasia (MPN) the development of fibrosis and increased vessel density correlate with poor prognosis. The JAK2V617F mutation constitutively activates JAK2, which phosphorylates signal transducer activator of transcription (STAT), up-regulating vascular endothelial growth factor (VEGF), which might be responsible for angiogenesis in MPN. Galectins are involved in the development of fibrosis and angiogenesis and might also be involved in activation of the JAK/STAT pathway in MPN. Methods: 106 MPN patients, 36 essential thrombocythemia (ET), 25 polycythemia vera (PV) and 45 primary myelofibrosis (PMF), were analyzed for the expression pattern of galectin-1, galectin-3, pSTAT3, pSTAT5 and MVD by immunostaining of bone marrow biopsy sections followed by automated image analysis. The JAK2 mutational status was analysed through real time PCR in blood samples. Results: The expression of galectin-1 was significantly higher in all MPN patients compared to normal controls. Galectin-3 was expressed more in PV patients. MVD was significantly higher in all MPN patients and correlated with galectin-1 and pSTAT5 expression. pSTAT5 expression showed a trend of higher expression in patients carrying the JAK2V617F mutation as well as in PV patients. PMF patients and all JAK2V617F positive patients showed a significantly higher pSTAT3 expression compared to control and ET patients. Conclusion: The findings suggest the involvement of galectin-1 in MPN development, regardless of the subtype. Furthermore involvement of galectin-3 in PV development, pSTAT5 in that of PV and JAK2V617F positive patients and angiogenesis, as well as pSTAT3 is involved in the pathogenesis of PMF.

Keywords: MPN, myeloproliferative neoplasia, galectin, JAK, STAT, angiogenesis, MVD

Introduction
Myeloproliferative neoplasia (MPNs) are clonal bone marrow stem cell disorders, characterized by proliferation of the myeloid, erythroid and/or megakaryocytic cell lineages resulting in increased numbers of granulocytes, erythrocytes and/or platelets in the peripheral blood. The three classical Philadelphia chromosome-negative (Ph-) MPNs are polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) [1, 2].

In patients with a MPN, fibrosis and increased vessel density correlate with poor prognosis [3, 4]. Galectins are involved in the development of both fibrosis [5, 6] and angiogenesis [7] in other organs, and therefore might be involved in MPN development.

Galectins mediate cell adhesion and stimulate cell migration, proliferation and apoptosis, through β-galactoside moieties on the cell surface interacting with integrins, laminin and fibronectin. Galectin-1 (gal-1) is involved in tumour angiogenesis and since increased microvessel density (MVD) has been reported in MPNs [8-10], gal-1 might be involved in the regulation of angiogenesis in MPN. Increased galectin-3 (gal-3) expression has been shown to be involved in liver fibrosis [5, 11]. Therefore, we studied the gal-1 and gal-3 expression in bone marrow trephines of Ph- MPNs.

The signal transducer and activator of transcription (STAT) proteins are activated via the JAK/
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Table 1. Clinical and laboratory findings of patients with ET, PV, PMF and the control group.

<table>
<thead>
<tr>
<th></th>
<th>Essential thrombocytopenia n=36</th>
<th>Polycythemia n=25</th>
<th>Primary myelofibrosis n=45</th>
<th>Control bone marrow n=36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/females</td>
<td>11/25</td>
<td>8/17</td>
<td>25/19</td>
<td>23/13</td>
</tr>
<tr>
<td>Age, y, mean (SD)</td>
<td>59 (17.70)</td>
<td>65 (13.56)</td>
<td>67 (10.73)</td>
<td>56 (14.33)</td>
</tr>
<tr>
<td>JAK2 wild type/JAK2 mutation/JAK2 unknown</td>
<td>15/19/2</td>
<td>2/17/6</td>
<td>7/20/18</td>
<td>36/0/0</td>
</tr>
<tr>
<td>White blood cell count*10^9/L, mean (SD)</td>
<td>9.37 (2.89)</td>
<td>16.08 (12.22)</td>
<td>11.89 (11.38)</td>
<td>8.16 (4.44)</td>
</tr>
<tr>
<td>Minimum-maximum</td>
<td>4.4-18.30</td>
<td>5.70-62.00</td>
<td>0.90-70.60</td>
<td>2.80-23.80</td>
</tr>
<tr>
<td>Haemoglobin, mmol/L, mean (SD)</td>
<td>8.53 (1.26)</td>
<td>9.89 (1.81)</td>
<td>7.09 (1.58)</td>
<td>8.16 (1.10)</td>
</tr>
<tr>
<td>Minimum-maximum</td>
<td>6.10-12.00</td>
<td>6.70-13.50</td>
<td>3.30-10.60</td>
<td>6.10-11.10</td>
</tr>
<tr>
<td>Haematocrit L/L, mean (SD)</td>
<td>0.43 (0.06)</td>
<td>0.52 (0.09)</td>
<td>0.35 (0.08)</td>
<td>0.39 (0.05)</td>
</tr>
<tr>
<td>Minimum-maximum</td>
<td>0.29-0.61</td>
<td>0.37-0.68</td>
<td>0.15-0.50</td>
<td>0.30-0.52</td>
</tr>
<tr>
<td>Thrombocytes*10^9/L, mean (SD)</td>
<td>929 (346)</td>
<td>662 (316)</td>
<td>564 (532)</td>
<td>263 (137)</td>
</tr>
<tr>
<td>Minimum-maximum</td>
<td>327-1862</td>
<td>112-1371</td>
<td>15-2644</td>
<td>49-585</td>
</tr>
</tbody>
</table>

STAT pathway, by Janus Kinases (JAKs). A somatic mutation in the JAK2 gene, JAK2V617F, has been shown to be present in >95% of PV patients and in approximately 50% of ET and PMF patients [12, 13]. The JAK2V617F mutation disrupts the inhibitory function of the pseudokinase domain in the JAK2 gene, resulting in constitutively activation of JAK2 and phosphorylation of STAT5 [8-10, 14-16]. Phosphorylated STAT5 (pSTAT5) is known to be increased in PV patients [17, 18] and it was shown that activation of STAT3 induces up-regulation of vascular endothelial growth factor (VEGF) [19]. Therefore, we studied the JAK2 mutational status, pSTAT3 and pSTAT5 expression along with MVD in bone marrow trephines of patients with Ph-MPNs.

Materials and methods

Study population

The study was carried out on bone marrow trephines obtained from patients recorded at the Maastricht University Medical Centre, Maastricht, between January 1992 and December 2009, recorded at the Haga Hospital, The Hague, between January 2006 and December 2009 and recorded at the VieCuri Medical Centre, Venlo, between January 2005 and July 2010. The study was approved by the local institutional ethics committee. The study population consisted of 106 patients with a myeloproliferative neoplasm, with a mean age of 63.6 years at time of diagnosis (SD±14.7) ranging from 17 to 86 years. The patient population included in the study consisted of 36 ET (33.9%), 25 PV (23.6%), and 45 PMF (42.5%) patients. None of the patients received therapy when the biopsy was taken. All patients were clinically and histologically diagnosed according to the World Health Organization (WHO) 2008 classification [20] and independently reviewed by two pathologists. Of the patients 45 (42.5%) were men and 61 (57.5%) were women. Fifty-six patients were carriers of the JAK2V617F mutation (19 ET, 17 PV and 20 PMF patients), 24 patients were carriers of the JAK2 wild type (15 ET, 2 PV and 7 PMF patients) and of 26 patients the JAK2 mutational status was unknown, because of insufficient DNA to detect the JAK2 status by PCR or because the patients died prior to the availability of the JAK2V617F test (see Table 1). The patients were subdivided for the grading of myelofibrosis (mf) into mf 0/1 and mf 2/3; 43 patients belonged to the mf 0/1 group (19 ET, 12 PV, 12 PMF) of which 24 were JAK2V617F positive and 11 carried the JAK2 wild type gene and 61 belonged to the mf 2/3 group (17 ET, 12 PV, 32 PMF) of which 31 were JAK2V617F positive and 13 carried the JAK2 wild type gene.

The control group consisted of 36 morphologically normal negative staging biopsies from patients with non-Hodgkin lymphoma and Hodgkin lymphoma with a mean age of 55.8 years.

Immunohistochemistry

The bone marrow biopsy specimens were decalcified using the EDTA decalcification for four hours, followed by standard tissue processing...
and paraffin embedding. From the paraffin-embedded blocks 3μm sections were cut for immunohistochemical staining and mounted on starfrost slides (Knittel Gläser, Germany). All the antibodies were tested for specificity on positive and negative tumour control slides and also individually tested on decalcified control bone marrow biopsies, resulting in a variation of immunohistochemical techniques, optimised for all individual antibodies.

Antihuman galectin-1 (R&D systems, Minneapolis, MN) was used at a dilution of 1:500 and antihuman galectin-3 (R&D systems, Minneapolis, MN) at a dilution of 1:50. After deparaffinization and blocking of endogenous peroxidase activity (0.3% H₂O₂ in methanol) antigen retrieval was performed by boiling in citric acid (pH 6.0) for 10 minutes in a water bath of 100°C. After blocking with 5% bovine serum albumin/phosphate buffered saline (BSA/PBS), primary antibody was applied in 0.5% BSA/PBS. Slides were then incubated with a biotin-labelled secondary antibody (gal-1: polyclonal swine anti-rabbit, Dako (Glostrup, Denmark) and gal-3: rabbit anti-goat, Dako (Glostrup, Denmark) at a dilution of 1:200 and 1:500 respectively for 30 minutes. Staining was performed with the StrepABComplex/HRP kit (Dako, Glostrup, Denmark) according to the manufacturer’s instructions. After developing the colour with freshly made diaminobenzidine solution (Dako, Glostrup, Denmark), slides were counterstained with haematoxylin (Merck, Whitehouse Station, NJ), dehydrated and mounted in Entellan (Merck).

Immunohistochemical staining of pSTAT3 and pSTAT5 was carried out using the antihuman rabbit monoclonal antibody pSTAT3 (Tyr705) and pSTAT5 (Tyr694) at a dilution of 1:50 and 1:200 respectively (Cell signaling Technology, Danvers, MA). After deparaffinization and antigen retrieval by boiling for 20 minutes in 1 mM Tris EDTA pH 8.0 in a warm water bath, endogenous peroxidase activity was blocked in 3% H₂O₂ in methanol. After blocking with blocking solution (Tris Buffered Saline Tween (TBST) with 5% horse serum), primary antibody was applied in TBST with 5% horse serum (pSTAT3) and TBST with 1% BSA (pSTAT5) overnight. The slides were then incubated with powervision (Immunologic, Duiven, The Netherlands) for 40 minutes. Development of the colour and counterstaining as described above.

The 142 trephines (MPN patients plus control patients) were immunohistochemically analysed using an automated immunostainer (Dako autostainer Link 48) with CD34 (clone QBend 10, Dako). CD34 was incubated for 20 minutes at room temperature. The reaction was revealed by means of the Dako Envision Flex Kit (Dako) according to the manufacturer’s instructions.

Quantification of staining

Gal-1, gal-3, pSTAT3 and pSTAT5 staining (see Figure 1) was quantified using an image processing and analysis system (Leica, Cambridge, UK) linked to a Leica DML3000 light microscope (Leica Quantimet, Germany). The program used in this system was QWin (Leica’s Windows-based image analysis tool-kit-Leica, Cambridge, UK). The surface area of galectin present was measured separately in cell nuclei and in stroma. All measurements were conducted at 40x magnification, in minimal three to maximal five complete hot spot bone marrow fields per slide, to measure total tissue, total cytoplasmic area positive and negative staining (gal-1 and gal-3), total nuclei positive (pSTAT3 or pSTAT5) and total nuclei count. The amount of positivity was calculated as the percentage of the total tissue area (gal-1 and gal-3) or the percentage of positive nuclear pixels related to the total number of nuclear pixels (pSTAT3 and pSTAT5).

MVD was assessed by counting the number of CD34 positive capillary-, arteriolar- or sinusularmen in five 1 mm² fields at 100x magnification, calculating the mean over these five fields.

The grading of fibrosis was done according to the European consensus on grading of bone marrow fibrosis [21].

To validate the data obtained at the molecular level, we tried to isolate DNA from bone marrow biopsies. However, the quality of the DNA was very poor and the DNA was too fragmented to be used.

Statistical analysis

The data were statistically evaluated using the SPSS 15 statistical package, analyzed descriptively (descriptives, explore and crosstabs). Statistical comparison was performed by Mann-Whitney U-test when comparing medians. Differ-
ences were considered significant when *p*-value was less than 0.05. Pearson’s test was performed for correlating the expression of gal-1 with MVD, gal-3 with MVD, pSTAT3 with MVD and pSTAT5 with MVD.

For the analysis of pSTAT5, bone marrow of the Haga hospital, The Hague, was withdrawn, due to inappropriate staining of the bone marrow. Only 30 ET patients, 16 PV and 34 PMF patients and a total of 20 control bone marrows were available for pSTAT5 analysis.

In some cases bone marrow tissue was lost during the pre-treatment of the slides; for gal-1 we report 1 missing value, for pSTAT5 6, and for MVD 5 missing values. For the grading of myelofibrosis we report 2 missing values.

**Results**

The results of all staining percentages are summarized in Table 2 and 3. Qualitative microscopic evaluation of gal-1 staining showed its expression mainly in the immature myeloid cell component. A weak expression of gal-1 was seen in the cytoplasm of the megakaryocytes, no expression of gal-1 was seen in the erythroid cell line. Gal-1 was expressed significantly more in bone marrow of PMF patients compared to the control slides (*p*=0.036). The mean percentage of gal-1 for all MPN patients together was 7.8% and 6.3% for the control patients (*p*=0.027). The expression between gal-1 and MVD was significantly correlated (*p*=0.007). Gal-3 was present in immature and mature myeloid cells and was only weakly expressed in megakaryocytes, endothelial cells and erythropoietic cells. Statistical analysis of gal-3 revealed a significant difference between PV and ET patients (*p*=0.019) and between PV and PMF (*p*=0.044) patients, with higher gal-3 expression in PV patients. There was no significant correlation between gal-3 and MVD and no significant difference between patients with different JAK2
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pSTAT3 was localized in immature and mature myeloid cells and in endothelial cells. In the evaluated bone marrow biopsy trephines, the percentage of pSTAT3 was higher in JAK2V617F positive patients compared to patients with wild type JAK2 (p=0.018). There was also a significant correlation between pSTAT3 and MVD (p=0.000).

pSTAT5 was expressed in immature myeloid cells, the nuclei of adipocytes, some endothelial cells and in the nuclei of megakaryocytes and partly a weak expression in the cytoplasm of megakaryocytes. pSTAT5 was significantly correlated with the MVD (p=0.020). No statistically significant difference but a trend was reached between patients carrying the JAK2V617F mutation and patients without the mutation as well as in PV patients compared to ET and PMF patients.

In the total MPN group the mean MVD was sig-mutational status.

Table 3. Percentage of gal-1, gal-3, pSTAT3 and pSTAT5 in JAK2 positive and JAK2 negative patients.

<table>
<thead>
<tr>
<th>JAK2 positive n=56</th>
<th>JAK2 negative n=24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galectin-1, %*, mean (SD)</td>
<td>8.50 (4.04)</td>
</tr>
<tr>
<td>Minimum-maximum (CI)</td>
<td>1.12-20.32 (7.41-9.59)</td>
</tr>
<tr>
<td>Galectin-3, %*, mean (SD)</td>
<td>8.93 (5.34)</td>
</tr>
<tr>
<td>Minimum-maximum (CI)</td>
<td>0.56-23.50 (7.49-10.37)</td>
</tr>
<tr>
<td>pSTAT3, %#, mean (SD)</td>
<td>5.53 (2.80)</td>
</tr>
<tr>
<td>Minimum-maximum (CI)</td>
<td>1.02-14.67 (4.77-6.29)</td>
</tr>
<tr>
<td>pSTAT5, %#, mean (SD)</td>
<td>4.22 (3.48)</td>
</tr>
<tr>
<td>Minimum-maximum (CI)</td>
<td>0.26-13.71 (3.02-5.41)</td>
</tr>
<tr>
<td>MVD, 1 mm², mean (SD)</td>
<td>52.77 (30.58)</td>
</tr>
<tr>
<td>Minimum-maximum (CI)</td>
<td>3.60-122.40 (44.72-63.18)</td>
</tr>
</tbody>
</table>

* calculated as percentage positive area of total tissue area. # calculated as percentage positive nuclei of total nuclei count.

Table 2. Percentage of gal-1, gal-3, pSTAT3 and pSTAT5 in ET, PV, PMF, all MPN patients and control patients.

<table>
<thead>
<tr>
<th>Essential thrombocythemia n=36</th>
<th>Polycythemia vera n=25</th>
<th>Primary myelofibrosis n=45</th>
<th>All MPN patients n=106</th>
<th>Control patients n=36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galectin-1, %*, mean (SD)</td>
<td>7.80 (4.37)</td>
<td>8.15 (4.50)</td>
<td>7.70 (3.35)</td>
<td>7.84 (3.97)</td>
</tr>
<tr>
<td>Galectin-3, %*, mean (SD)</td>
<td>5.90-9.49 (5.69-10.09)</td>
<td>6.27-8.95 (6.76-8.65)</td>
<td>8.15 (5.43)</td>
<td>4.23-8.57</td>
</tr>
<tr>
<td>pSTAT3, %#, mean (SD)</td>
<td>5.19 (4.21)</td>
<td>4.18 (2.18)</td>
<td>7.72 (5.90)</td>
<td>8.85 (5.41)</td>
</tr>
<tr>
<td>pSTAT5, %#, mean (SD)</td>
<td>4.16-11.77 (2.77-7.63)</td>
<td>3.98-6.42 (3.99-5.49)</td>
<td>5.52 (3.29)</td>
<td>2.67-5.17</td>
</tr>
<tr>
<td>MVD, 1 mm², mean (SD)</td>
<td>3.40-89.60 (37.72-22.18)</td>
<td>3.50-11.77 (4.72-3.58)</td>
<td>0.00-13.71 (4.21-4.21)</td>
<td>1.18-9.29</td>
</tr>
<tr>
<td>Minimum-maximum (CI)</td>
<td>3.40-122.40 (29.70-49.07)</td>
<td>5.80-111.20 (35.77-70.80)</td>
<td>12.80-122.40 (50.30-76.39)</td>
<td>17.94-34.27</td>
</tr>
<tr>
<td>Minimum-maximum (CI)</td>
<td>3.40-122.40 (44.72-63.18)</td>
<td>3.40-122.40 (44.72-63.18)</td>
<td>17.20-111.20 (40.64-65.24)</td>
<td>17.94-34.27</td>
</tr>
</tbody>
</table>

* calculated as percentage positive area of total tissue area. # calculated as percentage positive nuclei of total nuclei count.
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significantly higher compared to the control group \( (p=0.000) \) and the MVD was significantly higher expressed in PV \( (p=0.006) \) and PMF \( (p=0.000) \) patients compared to the control group. ET patients compared to PMF patients showed also a statistically significant difference with a higher MVD expression in PMF patients \( (p=0.003). \) PMF patients showed higher MVD (58.5 vessels/mm\(^2\)) than ET (37.7 vessels/mm\(^2\)) and PV patients (47.6 vessels/mm\(^2\)). Comparing the \( JAK2^{V617F} \) positive patients to the \( JAK2^{V617F} \) negative patients the MVD was not significantly different.

Concerning the myelofibrosis grading and the stainings we report a statistically significant higher gal-1 \( (p=0.013) \) and gal-3 \( (p=0.012) \) expression in the mf 0/1 group compared to the mf 2/3 group. For MVD there was a higher expression of MVD in the mf 2/3 group \( (p=0.001) \) compared to the mf 0/1 group and also the Pearson correlation showed a significant correlation of MVD with the grading of myelofibrosis \( (p=0.000). \)

Discussion

In this study, the expression of gal-1, gal-3, pSTAT3 and pSTAT5 along with the MVD in bone marrow cells was immunohistochemically measured in ET, PV, PMF and control bone marrows.

Gal-1 is known to be involved in tumour angiogenesis [7]. The higher expression of gal-1 and MVD in the total group of MPN patients in our study together with a significant correlation between gal-1 and MVD, suggests a role of gal-1 in the increased angiogenesis in MPN patients. These results assign a possible target for the angiogenesis inhibitor anginex, as gal-1 was identified as receptor for anginex. Anginex blocks the adhesion and migration of angiogenically activated endothelial cells, leading to apoptosis and inhibition of angiogenesis [22]. In gal-1-null mice treatment with anginex did not inhibit tumour growth in contrast to the wild type mice where tumour growth and vessel density was significantly inhibited with anginex treatment [7].

Increased expression of gal-3 has been associated with liver fibrosis secondary to diverse types of injury [11]. However, in the mf 0/1 group we saw a higher gal-3 expression compared to the mf 2/3 group. Also we saw no significant correlation between gal-3 and MVD. These findings contradict the relation between increasing fibrosis, MVD and gal-3 expression in MPN trephines. On the other hand we were able to show higher gal-3 expression in PV patients. Recently, it was also demonstrated that gal-3 is predominantly expressed in Chronic Myeloid Leukemia (CML) cells, where gal-3 expression support the molecular signalling pathways for maintaining CML in the bone marrow and resistance to therapy [23, 24]. Therefore there are indications that gal-3 might play a role in MPN pathogenesis.

Constitutive activation of STAT proteins is present in a variety of haematological disorders [25-29]. STAT3 activation has been reported in PV and ET and low pSTAT3 levels in PMF patients [17, 30]. However, our study does not confirm these results, possibly due to a relative high amount of PMF patients and lower amounts of PV and ET patients.

Activated STAT3 has an important role in the regulation of megakaryopoiesis and thrombopoiesis in vivo, via activation of Bcl-xL inhibiting apoptosis of megakaryocytes [31]. The bone marrow of PMF patients is characterized by a proliferation of the megakaryocytic cell line. The megakaryocytes often demonstrate dense clustering with cloud like nucleus [20]. The increased megakaryocytes with deviated forms in the bone marrow of PMF patients might be due to the decreased megakaryocyte apoptosis as result of increased STAT3 activation in PMF patients. The higher pSTAT3 expression in \( JAK2^{V617F} \) positive patients indicates an increased STAT3 activation generated by the presence of the \( JAK2^{V617F} \) mutation. In diverse cancer types it was shown that constitutive activation of STAT3 induces vascular endothelial growth factor (VEGF) expression [19]. In our study we show a correlation between pSTAT3 and MVD, indicating that the increased MVD seen in MPN patients, especially in PMF patients, might be induced by the constitutive activation of STAT3 resulting in increased expression of VEGF.

Our finding of higher pSTAT5 expression in PV and \( JAK2^{V617F} \) positive patients is in line with earlier published data [14, 17, 32, 33]. This indicates that the presence of the \( JAK2^{V617F} \) mutation generates increased levels of pSTAT5. However, in our study the pSTAT5 expression
Galectins in myeloproliferative neoplasia did not reach statistical significant difference but only showed a trend between patients carrying the JAK2V617F mutation and patients without the mutation as well as in PV patients compared to ET and PMF patients. This might be due to the high number of patients with an unknown JAK2 status and also to the small PV patient population. The correlation between pSTAT5 and MVD might suggest other pathways involved in the increased MVD seen in MPN patients. pSTAT5 can interact with p85, a regulatory subunit of PI3K/Akt pathway, and might increase VEGF via the PI3K/Akt and mammalian target of rapamycin (mTor) pathway as was already shown in chronic myeloid leukaemia (CML) [34-36].

In line with other studies[37, 38], we found the bone marrow MVD in the total MPN group and in PV and PMF patients to be significantly higher compared to the control group. The increased MVD reflects increased angiogenic activity which might be induced by hypoxia, via hypoxia-inducible factor (Hif) and VEGF, or by normoxia, directly via VEGF.

Regarding the MVD and fibrosis in MPN patients, Boveri et al. [39] found a higher MVD along with a higher grading of fibrosis, which is line with our study. Other studies showed higher MVD in PMF, post-ET myelofibrosis and post-PV myelofibrosis patients compared to ET and PV patients indicating that angiogenesis is primarily involved in later stages of the disease [38-41].

In conclusion, the characteristic megakaryopoietic abnormalities and also the higher MVD expression in PMF trephines can be explained by a higher pSTAT3 expression in PMF patients. Also gal-1 expression is correlated with the MVD with anginex as potential new therapy for MPN patients. pSTAT5 expression showed a trend of higher expression in PV and JAK2V617F positive patients, possible induced by the JAK2V617F mutation and also gal-3 expression seems correlated with PV. Further, the increased MVD expression in MPN patients with higher myelofibrosis grading suggests the important role of angiogenesis in the development of myelofibrosis.

Based upon these data we support the concept that the microenvironment plays an important role in haematological malignancies [42, 43]. Interactions between stroma and haematopoietic cells in MPNs constitute possible targets for therapy.

Address correspondence to: Dr. Koopmans SM, Department of Pathology, Maastricht University Medical Centre Postbus 5800, 6202 AZ Maastricht, The Netherlands Tel: +31-(0)433874641; E-mail: s.koopmans@mumc.nl

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