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Early tumor detection and intervention are important determinants of survival in patients with cancer. We have recently reported that the “platelet angiogenesis proteome” may be used to detect microscopic tumors in mice. We now present evidence that changes in platelet-associated platelet factor-4 (PF-4) detect malignant growth across a spectrum of human cancers in mice. A deregulated expression of an 8206-Da protein was observed by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-ToF MS) proteomic comparison of platelets from normal and tumor-bearing mice. The differentially expressed protein was identified as PF-4 by tandem mass spectrometry and ProteinChip immunoassay using anti–PF-4 antibody. The platelet-associated PF-4 appeared to be up-regulated in early growth of human liposarcoma, mammary adenocarcinoma, and osteosarcoma. A 120-day follow-up study of liposarcoma revealed a sustained 2-fold or higher increase of platelet-associated PF-4 at 19, 30, and 120 days. In contrast, only an insignificant change of PF-4 was observed in the plasma of mice bearing the different human tumor xenografts, and throughout the 120 days of the liposarcoma study. We conclude that platelet-associated PF-4, but not its plasma counterpart, may represent a potential biomarker of early tumor presence. (Blood. 2008;111:1201-1207)

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interaction of VEGFα with its receptor (VEGFR-2) thus inhibiting angiogenesis.34,36 A PF-4 derivative generated by peptide bond cleavage between Thr16 and Ser17 exhibits a 30- to 50-fold greater growth inhibitory activity on endothelial cells than PF-4 itself.37 The antitumor effect of PF-4 is also revealed by a decrease in the number and size of lung metastases of B16F10 melanoma18 and a decrease in growth of HCT-116 human colon carcinoma.39 PF-4 modifies the mitogenic effect of bFGF on fibroblasts,40 inhibits the proliferation of activated human T cells41 and tumor-infiltrating lymphocytes, and inhibits cytokine release by tumor stroma.42

Here we present new data demonstrating that changes in the platelet concentration of PF-4 may be used as a novel biomarker to detect human tumor xenografts that range from microscopic to macroscopic size. These tumors in mice include human liposarcoma, mammary adenocarcinoma, and osteosarcoma.

Methods

Human-tumor xenografts

All of the cancer cell lines used exhibit either nonangiogenic (microscopic, dormant tumors) or angiogenic (rapidly growing tumors) phenotypes in immunodeficient mice. The nonangiogenic and angiogenic cell line have been previously described by Folkman and colleagues (Almog et al22; Achilles et al44; and Naumann et al45). For each of the 3 parent cell lines (liposarcoma [SW872], osteosarcoma [KHOS-24OS], and mammary adenocarcinoma [MDA-MB-436]) 2 phenotypes exist, a nonangiogenic tumor and an angiogenic one.

All cell lines were cultured in DMEM containing 10% heat-inactivated fetal bovine serum (HyClone, Logan, UT), 1% antibiotics (penicillin, streptomycin), and 0.29 mg/mL 1-glutamine in a humidified 5% CO2 incubator at 37°C. For injections into mice, 80% to 90% confluent tumor cells were rinsed in phosphate-buffered saline (PBS; Sigma-Aldrich, St Louis, MO), briefly trypsinized and suspended in serum-free DMEM. Five million viable cells from each of the tumor cell lines were suspended in 200 μL serum-free media and implanted subcutaneously (SW872 and KHOS-24OS cell lines) into the flanks of 6- to 8-week-old male severe combined immunodeficient (SCID) mice. For the human breast adenocarcinoma (MDA-MB-436) cell line, 1 million viable cells were suspended in 50 μL serum-free media and implanted in the mammary fat pad through a 0.75- to 1-cm incision. The corresponding sham operation was a 0.75- to 1.0-cm incision. The mice were terminally bled under isoflurane anesthesia at 30 days after implantation to collect the platelets. The mice were obtained from the Massachusetts General Hospital (MGH; Boston, MA). Animals and tumors were monitored daily as per institutional guidelines. Data were analyzed using Student t test to determine the P values for differences between means; the graphs in Figures 4 and 5 are therefore expressed as means plus or minus SEM.

Platelet and plasma processing for SELDI-ToF mass spectrometry

Blood samples were processed according to standard methods for platelet collection. Briefly, mice were anesthetized using 2% isoflurane/1 L O2 flow system. Whole blood (1 mL) was collected by terminal cardiac bleed into 105 mM sodium citrate (pH 5) anticoagulant at a ratio of 1:9 (vol/vol) buffer to blood. The first centrifugation step at 180g for 20 minutes at room temperature allowed for the collection of platelet-rich plasma (PRP). A second centrifugation at 900g for 30 minutes at room temperature separated the platelets and the upper phase, platelet-poor plasma (PPP). This resulted in 2 separate phases for processing and analysis by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-ToF MS) technology (Ciphergen Biosystems, Fremont, CA), platelet pellets, and PPP. The pellets and 20 μL PPP from each mouse were processed in 25 μL and 40 μL, respectively, U9 buffer (2% CHAPS [3-[3-cholamidopropyl] dimethylammonio]-1-propanesulfonate), 50 mM Tris-HCl, pH 9; Ciphergen Biosystems) for 1 hour at room temperature. Platelet lysates were then centrifuged at 10 000g for 1 minute at 4°C. Both platelet extracts and PPP were fractionated by anion-exchange chromatography modified after the expression difference mapping (EDM) serum fractionation protocol (Ciphergen Biosystems). The fractionation was performed in a 96-well format filter plate on a BioScreen 2000 Laboratory Work Station (Beckman Coulter, Fullerton, CA) equipped with a Micromix 5 shaker (Siemens Medical Solutions Diagnostics, Deerfield, IL). An aliquot of 20 μL platelet and 60 μL denatured plasma diluted with 100 μL 50 mM Tris-HCl (pH 9) was transferred to a filter-bottom 96-well microplate prefiltered with Q Ceramic HyperD F sorbent beads (Pall, East Hills, NY) rehydrated and pre-equilibrated with 50 mM Tris-HCl (pH 9). All liquids were removed from the filtration plate using a multiscreen vacuum manifold (Millipore, Bedford, MA) into respective wells of 96-well microtiter plates with the capture of the initial flow-through as fraction 1. This step was repeated for subsequent incubations with 2 × 100 μL of the following buffers: pH 7.5 (1 M urea, 0.1% CHAPS, 50 mM NaCl, 2.5% acetonitrile, 50 mM Tris-HCl [50 mM HEPES];) pH 5 (1 M urea, 0.1% CHAPS, 50 mM NaCl, 2.5% acetonitrile 50 mM NaAcetate); pH 4 (1 M urea, 0.1% CHAPS, 50 mM NaCl, 2.5% acetonitrile 50 mM NaAcetate); pH 3 (1 M urea, 0.1% CHAPS, 500 mM NaCl, 2.5% acetonitrile 50 mM NaCitrate), which yielded the respective fractions 2, 3, 4, and 5. A final organic wash with 33% isopropanol/16.7% acetonitrile/8% formic acid represents fraction 6.

Expression difference mapping (EDM) on ProteinChip arrays was carried out using weak cationic exchange chromatography protein arrays (WCX2 and CM10 ProteinChip arrays; Ciphergen Biosystems) by loading sample fractions onto a 96-well bioprocesor, and equilibrating with 50 mM sodium acetate 0.1% octyl glucoside (Sigma), pH 5. A further dilution of 40 μL anion exchange chromatography fraction into 100 μL of the same buffer on each array spot was incubated for 1 hour. Array spots were washed for 3 minutes with 100 μL 50 mM sodium acetate 0.1% octyl glucoside (pH 5). After rinsing with water, 1 μL sinapinic acid matrix solution was added twice to each array spot. For protein profiling, all fractions were diluted 1:2.5 in their respective buffers used to pre-equilibrate ProteinChip arrays. This step was followed by readings using the Protein Biology System II (PBSII) and Protein Ciphen System 4000 (PCS4000) SELDI-ToF mass spectrometer (Ciphergen Biosystems) and processed with the ProteinChip Software Biomarker Edition, Version 3.2.0 (Ciphergen Biosystems). After baseline subtraction, spectra were normalized by a total ion current method. Peak detection was performed using Biomarker Wizard software (Ciphergen Biosystems) using a signal-to-noise ratio of 3.

For immunocapture experiments, anti–PF-4 antibody (rabbit, affinity-purified polyclonal antibody; R&D, Minneapolis, MN) was immobilized on preactivated ProteinChip array (RS100; Ciphergen Biosystems). After blocking and washing of excess antibody, platelet extract diluted in BSA Triton X100 PBS was incubated with the immobilized antibody. After washing with PBS containing urea and CHAPS, the captured proteins were detected by SELDI. To confirm a full capture of the protein, the mobile phase was also incubated on a preactivated spot for a second time to verify immunodepletion. A final confirmation of the protein identity was obtained using murine PF-4 enzyme-linked immunosorbent assay (ELISA; data included as Figure S2).

Protein identification

Candidate protein biomarker was purified by affinity chromatography on beads with immobilized IgG spin columns and by reverse-phase chromatography. The purity of each step was monitored using normal phase (NP) ProteinChip arrays. The enriched fractions were reduced by 5 mM DTT in Tris-HCL buffer (pH 9) and alkylated with 25 mM iodoacetamide. The alkylated preparation was finally purified using 16% tricine sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The gel was stained by Colloidal Blue Staining Kit (Invitrogen, Carlsbad, CA). Selected protein bands were excised, washed with 200 μL 0.5% methanol/10% acetic acid for 30 minutes, dehydrated with 100 μL acetonitrile (ACN) for 15 minutes, and extracted with 70 μL 50% formic acid, 25% ACN, 15% isopropanol, and 10% water for 2 hours at room temperature with vigorous shaking. The candidate biomarkers in extracts were again verified by
Table 1. Amino acid sequences of identified peptides

<table>
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<th>m/z</th>
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<th>Mowse score</th>
<th>Significant homology score</th>
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<td>&gt;17</td>
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<tr>
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<td>HCAVPQLIATLKNGR + CAM‡</td>
<td>14</td>
<td>&gt;13</td>
<td>&gt;15</td>
</tr>
</tbody>
</table>

*Ion score is $-10^{Log(P)}$, where P is probability that the match is a random event.
†Individual ion scores higher than a number displayed in the column indicate significant homology.
‡Individual ion scores higher than a number displayed in the column indicate identity or extensive homology ($P < .05$).
§Carbamidomethyl cysteine.

analysis of 2 μL on a normal phase ProteinChip array (NP20). The remaining extract was digested with 20 μL of 10 ng/μL modified trypsin (Roche Applied Science, Indianapolis, IN) in 50 mM ammonium bicarbonate (pH 8) for 3 hours at 37°C. Single MS and MS/MS spectra were acquired on a QSTAR mass spectrometer (Applied Biosystems, Foster City, CA) equipped with a Ciphergen PCI-1000 ProteinChip Interface. A 1-μL aliquot of each protease digest was analyzed on an NP20 ProteinChip array in the presence of CHCA matrix (Ciphergen Biosystems). Spectra were collected from m/z values of 900 to 3000 in single MS mode. After reviewing the spectra, specific ions were selected and subjected to collision-induced dissociation (CID). The CID data were submitted to the database-mining tool Mascot (Matrix Science, Boston, MA) for identification (Table 1; Figure S1, available on the Blood website; see the Supplemental Materials link at the top of the online article).

Results

Identification of the differentially expressed PF-4 in tumor-bearing mice

Platelet lysates from healthy non–tumor-bearing mice and those bearing nonangiogenic or angiogenic xenografts of human liposarcoma, mammary adenocarcinoma, and osteosarcoma for a minimum of 30 days were subjected to a standard biomarker discovery protocol. Among the several unknown differentially expressed proteins, elevation was observed in the platelet content of a polypeptide with an apparent molecular weight of 8206 Da (Figures 1,2). This protein was later found to be consistently elevated across 3 repeated experiments, and across several tumor types.

A careful analysis was therefore performed to identify the protein of interest. The 8206-Da protein was purified using chromatography and SDS-PAGE. Gel-purified protein was digested with trypsin, and unique tryptic fragments were analyzed by tandem MS (data in Figure S1). The ion with m/z of 1350.75 was identified as Cys-carbamidomethylated peptide HCAVPQLIATLK with Mowse score46 of 60 (ion score $> 17$ indicated significant homology; $> 22$ indicated identity or extensive homology). The ion with m/z of 1677.90 was identified as Cys-carbamidomethylated peptide HCAVPQLIATLKNGR with Mowse score of 14 (ion score $> 13$ indicated significant homology; $> 15$ indicated identity or extensive homology). Both peptides corresponded to unique tryptic fragments of mouse PF-4 (SwissProt accession no. Q9Z126) previously identified.47-49 Theoretic molecular weight of mouse PF-4 is 8210.71 Da, however considering 2 Cys-Cys bridges in the polypeptide molecule, the expected MW is 8206.71 Da. The latter value is very close to the observed experimental molecular weight of the candidate biomarker.

Confirmation of identity of platelet-derived PF-4 by ProteinChip immunoassay using anti–PF-4 antibody

Further validation of this candidate biomarker was obtained by immunoprecipitation using rabbit anti–PF-4 antibody. Figure 2 represents the spectral readout obtained from arrays coated with an immobilized anti–PF-4 antibody prior to incubation with the platelet extracts from mice. The presence of a thick protein peak at 8206 Da (arrow) validates both the presence and theoretic mass of PF-4 (Figure 2). The identity of the differentially expressed protein was further confirmed by immuno-capture/immunodepletion of the protein. The protein captured using the PF4-specific antibody has a peak identical to that of the recombinant protein (upper 2 panels of Figure 3), and the peak is absent in the mobile phase of the spotted lysate (Figure 3).

Figure 1. Identification of the platelet- and plasma-derived candidate proteins. Platelets were harvested from mice bearing nonangiogenic or angiogenic xenografts of human liposarcoma at 30 days after tumor implantation and compared with those of their littermate controls using a standard SELDI-ToF biomarker discovery method. A spectral readout from SELDI-ToF MS is presented here in gel view format, and groups are color-coded for clarity. Gray represents protein content of platelets from control animals; blue, from mice bearing the nonangiogenic dormant clone; and red, from mice bearing the angiogenic clone. A differentially expressed protein was observed at 8206 Da. The candidate peptide (**) was later analyzed and identified as PF-4. Each horizontal strip represents an individual mouse sample (n = 5), and the color intensity corresponds to the height of the protein peak. The experiment was reproduced on 2 independent occasions for a total of 15 mice per group.

Figure 2. Validation of candidate biomarker by immunoprecipitation. Spectral readout in gel view format obtained from arrays prepared with an anti–PF-4 antibody prior to incubation with platelet extracts from mice within the indicated groups. The labeled arrow indicates both the presence and theoretic mass of PF-4.
Validation of PF-4 as a surrogate marker of tumor presence

Platelets of nonangiogenic or angiogenic human liposarcoma xenografts, SW872, exhibited a 7-fold elevation of platelet-derived PF-4 compared with non–tumor-bearing controls at 30 days after implantation (Figure 4A) without a corresponding increase in PF-4 in the plasma. In this model, platelets of mice bearing the nonangiogenic xenografts (tumors less than 1 mm) contained PF-4 levels comparable with its angiogenic counterpart. Platelets of mice bearing the angiogenic mammary adenocarcinoma, MDA-MB-436 (Figure 4B), or the angiogenic osteosarcoma, KHOS-24OS (Figure 4C), revealed similar trends at 4- and 2-fold up-regulation, respectively. The nonangiogenic xenografts did not show an elevation of PF-4 in platelets. The platelet-associated PF-4 content was consistently found to be higher than that of the corresponding plasma, even though the degree of elevation varied based on tumor type (Figure 4A-C). Angiogenic tumors were associated with the greatest differences between platelet-derived PF-4 versus that of plasma, even though platelets of mice bearing the nonangiogenic tumors of liposarcoma also showed PF-4 elevation. Liposarcoma xenografts exhibited the greatest platelet content of PF-4, while the increases in PF-4 for mammary adenocarcinoma and osteosarcoma were not as large (Figure 4A-C).

Platelet PF-4 in early tumor detection

To test whether platelet content of angiogenesis regulators can be used in detection of early tumor growth, we explored the ability of PF-4 to predictably detect nonangiogenic (dormant) microscopic tumors in mice over an extended period of time. We conducted a time-course analysis of platelet-associated PF-4 in mice bearing a subcutaneous xenograft of the nonangiogenic (dormant) clone of human liposarcoma (SW872). The malignant progression of liposarcoma has been previously described, and it is known that the nonangiogenic (dormant) clone undergoes a spontaneous switch to the angiogenic phenotype, begins to grow, and becomes detectable by gross examination at a median of approximately 133 days after implantation. We show that PF-4 remains significantly elevated throughout a period of 120 days of observation of the nonangiogenic (dormant) state. Even without a palpable tumor, at 19 days, the median level of PF-4 in platelets is 1.7-fold higher than baseline without a corresponding increase in plasma level of the protein (Figure 5A,B). The plasma and platelet levels of PF-4 were similar at the time of implantation. However, while platelet PF-4 rose in the first 2 weeks of tumor growth, and remained elevated for the duration of the 120 days of the experiment, plasma PF-4 continued to decline (Figure 5B). The size of the tumor did not exceed 1 mm for the duration of the experiment.

Discussion

Numerous angiogenesis regulatory proteins are present in platelets. While the relative concentrations of these proteins remain stable under physiologic conditions, their levels change significantly in the presence of a tumor. There are other reports documenting angiogenesis regulatory factors in platelets of cancer patients, and continuing controversy persists as to whether serum or plasma levels of angiogenesis factors are more accurate for the measurement of angiogenesis-related diseases.

In a study of paired serum and plasma samples, VEGF levels correlated with platelet count in 116 patients with colorectal cancer, but not in controls. Support can be found for both serum or plasma measurements.

The diagnostic use of angiogenic proteins such as VEGF, bFGF, or PF-4 in early disease has been hindered in part by the minute levels of the proteins and their short half-lives in the circulation. The finding of platelet sequestration of angiogenesis regulatory proteins suggests a new modality for early detection of human cancer. We propose that angiogenesis regulators are not released from the mobile phase (fourth panel). For immunocapture experiments, anti–PF-4 antibody was immobilized on a preactivated ProteinChip array, followed by incubation with platelet extracts derived from mice bearing the dormant clone of liposarcoma. Comparison of a profile generated by the recombinant PF-4 (first panel) with that generated by platelet lysates of dormant liposarcoma-bearing mice reveals an identical molecular weight and isoelectric point of the protein in question (second panel). Immunodepletion of the PF-4 protein is confirmed by the absence of its respective peak from the mobile phase (fourth panel).
We introduce PF-4 as one of the platelet-associated angiogenesis regulators that may serve as an early tumor biomarker. We show that platelet-associated PF-4 can be detected as early as 19 days after implantation, and that a steady elevation of the protein can be observed throughout 120 days (Figure 5). While this paper does not provide sufficient data to support a functional role of PF-4 in tumor angiogenesis, there is sufficient published evidence that PF-4 is an angiogenesis suppressor and a tumor growth suppressor.25,26,38,39,62-64 PF-4 may modulate tumor growth by modifying VEGF effects35 or by binding and neutralizing heparin65 and related sulfated glycosaminoglycans42 required for the binding of proangiogenic factors.30,40,66 The binding and neutralization of heparin down-regulates angiogenesis mainly by preventing the binding of other angiogenesis regulators to heparan sulfate in tissues and by interfering with VEGF and bFGF signaling pathways.67 The high levels of VEGF and bFGF secreted by the nonangiogenic clone of SW872 liposarcoma43 may be counterbalanced by PF-4 leading to tumor quiescence and dormancy. The increase in platelet-associated PF-4 in dormant (nonangiogenic) tumors may therefore be reflective of the functional inhibition of angiogenesis in liposarcoma, which secretes large amounts of VEGF and bFGF.43 A feedback loop may exist in animals bearing tumors, such that increased VEGF and bFGF induce megakaryocyte synthesis of PF-4. This is supported by the finding that tumors that do not secrete large amounts of VEGF and bFGF, such as the nonangiogenic clones of MDA-MB-436 mammary adenocarcinoma and the KHOS-24OS osteosarcoma,45 do not manifest a marked elevation of PF4 (Figure 4). These tumors may use other means of tumor growth suppression. PF-4 appears to be a marker of angiogenesis and was present in the platelets of all of the tested angiogenic tumor models.

The changes in platelet-associated PF4 may have the potential to convey valuable clinical information about the angiogenic potential of the tumor, and a serial measurement of platelet PF-4 levels may provide us with the ability to detect tumor progression in an otherwise healthy subject.

Numerous reports have suggested an active role of platelets in cancer progression58,69 and in tumor growth and metastasis.70-72 Most investigators assume that platelets act as a reservoir of angiogenic proteins that are released into the sera.53,73 However, we show here that platelets actively sequester select proteins in tumor-bearing animals, and that this process is distinct from the nonspecific uptake of proteins such as albumin. One of the main reasons previous proteomic analysis on platelets74-78 may not have detected the differential expression of angiogenesis-related proteins was because these studies analyzed normal platelets and not platelets of cancer patients. We report for the first time, to our knowledge, the changes in the “platelet angiogenesis proteome” in response to the presence of a tumor in experimental animals.

Platelet-associated PF4 may be a potential tumor biomarker. Platelet-associated PF-4 should be explored in other mouse models of cancer and in high-risk patient populations predisposed to early tumor progression due to mutations in APC, p53, PTEN, or BRCA1. If validated, it may improve our ability to intervene very early in recurrent cancer, keep cancers in a dormant stage, and possibly convert cancer into a chronic, more manageable disease.79,80 As biologic modifiers, including angiogenesis inhibitors, which are relatively less toxic, become available for cancer therapy, early treatment may be much more possible than it has been in the past. A long-term goal would be to “treat the biomarker” until it

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**Figure 4.** PF-4 in platelets of mice bearing human-tumor xenografts. Platelets of mice bearing xenografts of SW872 liposarcoma (A), MDA-MB-436 mammary adenocarcinoma (B), or KHOS-24 osteosarcoma (C) were analyzed using SELDI-ToF. The blue bars represent whole platelet extracts and brown bars represent plasma. The platelets of non–tumor-bearing control mice served as a reference for the endogenous levels of platelet- and plasma-derived PF-4. The control group is shared by all 3 experiments. Platelets of nonangiogenic or angiogenic human liposarcoma xenografts, SW872, exhibited a 7-fold elevation of platelet-derived PF-4 compared with non–tumor-bearing controls at 30 days after implantation (A) without a corresponding increase of PF-4 in the plasma. Platelets of mice bearing the angiogenic mammary adenocarcinoma, MDA-MB-436 (B), also had significant elevation of platelet but not plasma PF-4. In the case of angiogenic osteosarcoma, KHOS-24OS (C), a similar trend at 4- and 2-fold up-regulation can be observed, but the value did not reach significance. Each bar represents the mean peak intensities corresponding to the level of the protein (± SEM) of 5 to 10 mice per experiment. Student t test was used to compare means of the groups. Each experiment was repeated twice.

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Various cancers59,61 did not identify this marker. We emphasize that while only PF-4 is being presented here, other angiogenesis regulatory proteins sequestered in platelets may have the same diagnostic capacity and remain to be identified.
returns to normal, before the onset of symptoms of recurrent tumor and before the tumor can be anatomically located.

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Authorship

Contribution: D.C. wrote the initial draft of the paper and (along with T-T.Y.) executed, analyzed, and evaluated the mass spectrometry data; T-T.Y. performed, analyzed, and evaluated the mass spectrometry data and provided invaluable expertise with the SELDI ToF MS technology; N.B. executed in vitro data and assisted with animal experiments; V.N.P. performed the purification and identification of candidate biomarkers; J.P. and A.A.-S. have been instrumental in the development of a murine PF4 ELISA and quantified the PF-4 protein; G.N.N. provided the breast and osteosarcoma models; E.B. assisted with the animal experiments; N.A. designed and executed the liposarcoma experiment and advised on the dormancy models; J.E.I. contributed to the evaluation of the data and the paper revisions; J.F. provided mentorship for the team and expertise in preparation of the paper; G.L.K. designed and performed the research, provided guidance for the group, analyzed the data, and revised the original paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Platelets may serve up biomarkers

Bruce S. Sachais
In this edition of Blood, Hochhaus and colleagues report on the long-term outcomes of the single-arm phase II trial of standard dose imatinib in a large cohort (n = 532; primarily chronic phase) of CML patients who had failed interferon therapy and were a median of 34 months from diagnosis. The initial results of this trial were the basis for the initial Food and Drug Administration approval of imatinib in CML. Among the study cohort (64% with inadequate response to interferon), 57% achieved a complete cytogenetic response (64% with inadequate response to interferon), of whom 41% remained in that response through the 5-year time point. In addition, 61% remained in the chronic phase 6 years after enrollment in the trial. As in the IRIS trial, the quality of the cytogenetic response by 12 months was predictive of long-term risks of progression.

So how does the study by Hochhaus and colleagues help us further understand the impact of imatinib therapy in CML? First, the study clearly shows that even in pretreated patients, imatinib keeps many in an excellent progression-free state for a long period of time. Indeed, the 6-year overall survival rate of 76% easily trumps historical controls of 15% to 20%. Second, the rates of response and progression-free survival and the percentage of patients who remain on imatinib are all lower compared with the newly diagnosed group in the IRIS trial.

The differences between this current trial and the IRIS trial suggest that disease progression in CML is probably not solely a BCR-ABL-driven phenomenon and that inadequate (or in this case delayed) suppression of the tyrosine kinase may allow disease evolution. The latter observation forms the basis for the current research agenda in CML: Will higher initial doses of imatinib, or a second-generation inhibitor such as dasatinib or nilotinib, decrease rates of progression? How can BCR-ABL be best suppressed in patients with mutations (such as the T315I) that render them insensitive to current kinase inhibitors? What is the best way to monitor response to tyrosine kinase inhibition, and what thresholds should be established for altering therapy or considering allogeneic stem-cell transplantation? Unraveling the mechanism of disease progression is crucial for guiding CML therapy, elucidating further targeted therapy beyond tyrosine kinase inhibition, and may yield insight into mechanisms of disease progression in the other chronic myeloproliferative disorders.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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Comment on Cervi et al, page 1201

Platelets may serve up biomarkers

Bruce S. Sachais UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE

Biomarkers, biological measurements that identify or “mark” the presence of disease, are nothing new to medicine, as exemplified by the routine use of CK-MB and troponin measurements to identify patients who have suffered a heart attack. However, the current “omics” revolution, which allows the simultaneous measurement of hundreds or thousands of molecules from a single sample, has reinvigorated the search for novel biomarkers for a variety of diseases.

In this issue of Blood, Cervi and colleagues used a proteomic technique known as mass spectroscopy to examine the platelet proteome of mice injected with a variety of tumor cells, compared with saline-injected controls. Among the proteins that differed in tumor-bearing mouse platelets, these investigators identified platelet factor 4 (PF4) as a biomarker of a variety of tumor types, including liposarcoma, osteosarcoma, and adenocarcinoma. A significant change in plasma PF4 was not detected.

These studies challenge us to look beyond traditional fluid sources of disease markers (plasma, urine, and cerebrospinal fluid). By examining platelets, Cervi et al were able to identify PF4 as a cancer-associated protein, even though PF4 levels do not change in the plasma. The lack of value of plasma PF4 as a biomarker in this study is not unexpected, as PF4 readily cleared from the plasma by binding to abundant glycosaminoglycans in the vasculature, highlighting the value of nontraditional biological samples to aid in the diagnosis of disease.

While Cervi et al used mass spectroscopy to identify and quantify PF4 as a tumor biomarker, this technology is not widely available in clinical laboratories. The study demonstrates that increases in platelet PF4 can also be measured using the much simpler and more accessible method of ELISA. The use of ELISA in a place of mass spectroscopy enhances our ability to validate the usefulness of platelet PF4 as a biomarker for early detection of cancer.

Although it is not a requirement, since biomarkers may simply have a disease association, biomarkers may have pathophysiological relevance to the disease that they identify. In this case, increases in platelet PF4 may be involved in tumor growth, as PF4 is known to regulate angiogenesis and may inhibit tumor growth. As such, PF4 in platelets represents not only a potentially useful cancer biomarker, but a potential target for early cancer therapy as well.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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