13 year old Male

- Presents to PCP with headache, vision changes, and walk with limp
- Dx: high-risk medulloblastoma
- Tx: GTR, XRT and 8 cycles of CCNU, CDDP and VCR
- Jehovah’s Witness
  - Admitted every nadir for q8h tranexamic acid and q12h cyroprecipitate
Angiogenesis required for tumor growth and metastasis

- **Tumor is dormant**
  - Somatic mutation
  - Small avascular tumor

- **Angiogenic switch**
  - Tumor secretion of angiogenic factors stimulates angiogenesis
  - Rapid tumor growth and metastasis

Affected fields

• Oncology
  – Solid tumors
  – Hem malignancies

• Surgery
  – Wound healing
  – Diabetic ulcers

• Cardiology
  – Reperfusion following infarct

• Neonatology
  – Retinopathy of prematurity
Inhibitors of Angiogenesis used in oncology

**Monoclonal Antibody**
- Bevacizumab
- Cetuximab

**Small Molecule Tyrosine Kinase Inhibitors**
- Erlotinib
- Pazopanib
- Sorafenib
- Sunitinib
- Vandetanib

**Inhibitors of mTOR**
- Temsirolimus
- Everolimus

**Other**
- Interferon alfa
- Lenalidomide
- Thalidomide
Platelets in oncogenesis

Direct

- Immune evasion
- Tumor homing and adhesion to endothelium
- Differential release of pro- and anti-angiogenic factors from granules
- Increases vascular integrity / prevention of tumor hemorrhage

Indirect

- Endothelial cell migration
- Recruitment of bone marrow-derived cells
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Tumor-promoting

Platelet-derived growth factor
Basic fibroblast growth factor
Vascular endothelial growth factor proteins
Fibrinogen

<table>
<thead>
<tr>
<th>Location</th>
<th>Name</th>
<th>Function</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour promoting</td>
<td>Cell surface</td>
<td>GP Ib/IX: Firm platelet adhesion and aggregation; tumour cell metastasis</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>GP IIa</td>
<td>Platelet adhesion and recovery from vascular injury, especially under high \shear blood flow; tumour cell metastasis</td>
<td>112</td>
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<td></td>
<td>GP VI</td>
<td>Platelet adhesion, activation and aggregation; tumour cell metastasis</td>
<td>55</td>
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<tr>
<td></td>
<td>P-selectin</td>
<td>Stabilize platelet aggregation and leukocyte arrest on endothelium; tumour growth and metastasis</td>
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<tr>
<td></td>
<td>PAR4</td>
<td>Thrombin receptor that activates platelets; enhances metastasis</td>
<td>62</td>
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<tr>
<td></td>
<td>Gaq</td>
<td>α-subunit of G protein mediates signalling during platelet activation; enhances metastasis</td>
<td>53</td>
</tr>
<tr>
<td>α-granules</td>
<td>Prothrombin</td>
<td>Activated by tissue factor to convert fibrinogen to fibrin, procoagulant and tumour progression</td>
<td>25</td>
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<tr>
<td></td>
<td>SERPINE1</td>
<td>Inhibits plasminogen activation and fibrinolysis; tumour invasion and angiogenesis</td>
<td>157</td>
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<tr>
<td></td>
<td>Plasminogen</td>
<td>Protease that breaks down fibrin in blood clots</td>
<td>157</td>
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<tr>
<td></td>
<td>uPA</td>
<td>Serine protease that converts plasminogen to plasmin</td>
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<tr>
<td></td>
<td>EGF</td>
<td>Enhances cell growth, proliferation, differentiation and migration</td>
<td>9</td>
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<tr>
<td></td>
<td>TGFβ</td>
<td>Regulates inflammation; both enhancing and inhibitory roles in tumorigenesis and progression</td>
<td>160</td>
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<tr>
<td></td>
<td>PDE4D</td>
<td>β2-Adrenergic G protein, a positive regulator of platelet activation</td>
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</tr>
<tr>
<td></td>
<td>bFGF</td>
<td>Mitogenic, tumour progression and angiogenesis</td>
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<td></td>
<td>HGF</td>
<td>Tumour progression, metastasis and angiogenesis</td>
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<tr>
<td></td>
<td>IGEF-1</td>
<td>Mitogenic, tumour progression and angiogenesis</td>
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<tr>
<td></td>
<td>VEGF</td>
<td>Regulates vascular permeability and stimulates angiogenesis</td>
<td>43</td>
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<tr>
<td></td>
<td>ANGPT1</td>
<td>Maintains vascular integrity; tumour progression and angiogenesis</td>
<td>162</td>
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<tr>
<td></td>
<td>MMP1</td>
<td>Tissue remodelling and matrix degradation</td>
<td>106</td>
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<tr>
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<td>MMP2</td>
<td>Tissue remodelling, matrix degradation and tumour cell invasion</td>
<td>106</td>
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<tr>
<td></td>
<td>MMP9</td>
<td>Matrix degradation and tumour cell invasion; metastasis</td>
<td>104</td>
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<tr>
<td></td>
<td>Fibrinogen</td>
<td>Coagulation, precursor of fibrin clots, matrix proteins, platelet adhesion and aggregation; protects and adheres tumour cells in circulation</td>
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</tr>
<tr>
<td></td>
<td>Fibrinectin</td>
<td>Extracellular matrix protein, binds integrins, fibrin and collagen; tumour cell invasion and metastasis</td>
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<tr>
<td></td>
<td>PAF acetylhydrolase</td>
<td>Inflammation and haemostasis; lipid messenger</td>
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<tr>
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<td>S1P</td>
<td>Vascular permeability, cell invasion and survival</td>
<td>165</td>
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<tr>
<td></td>
<td>LPA</td>
<td>Cell proliferation, survival and migration</td>
<td>166</td>
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<tr>
<td></td>
<td>P-selectin</td>
<td>Cell adhesion molecule, proinflammatory, marker of platelet activation; tumour progression</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>CCL2</td>
<td>Macrophage chemokines; tumour cell invasion and tumour macrophage infiltration</td>
<td>154</td>
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<tr>
<td></td>
<td>CCL3</td>
<td>Proinflammatory cytokine</td>
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<tr>
<td></td>
<td>CCL4</td>
<td>Chemokine that recruits leukocytes during inflammation; tumour progression</td>
<td>88</td>
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<td>CCL7</td>
<td>Proinflammatory, macrophage chemokine</td>
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<td>Gro−α</td>
<td>Neutrophil chemokine and monocyte arrest</td>
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<td></td>
<td>CXCL5</td>
<td>Proinflammatory cytokine</td>
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<tr>
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<td>IL-8</td>
<td>Proinflammatory, leukocyte chemotractant and angiogenesis</td>
<td>156</td>
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<tr>
<td></td>
<td>CXCL12</td>
<td>Chemokines; tumour progression and metastasis</td>
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<td>BDNF</td>
<td>Neuronal development and function; receptor TRKB supports tumorigenesis and metastasis</td>
<td>158</td>
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<tr>
<td></td>
<td>TP</td>
<td>Tumour progression and angiogenesis; chemotherapy activation</td>
<td>159</td>
</tr>
<tr>
<td>Dense granules</td>
<td>Serotonin</td>
<td>Platelet aggregation, vascular tone and cardiac functions; tumour angiogenesis and metastasis</td>
<td>124</td>
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<tr>
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<td>Histamine</td>
<td>Proinflammatory, increases vascular permeability</td>
<td>161</td>
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<tr>
<td></td>
<td>ATP</td>
<td>Triggers calcium influx and amplifies platelet aggregation</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>ADP</td>
<td>Potent activator of platelets, adhesion and aggregation</td>
<td>22</td>
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<tr>
<td></td>
<td>IL-1β</td>
<td>Proinflammatory cytokine induces angiogenesis</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>VEGF</td>
<td>Regulates vascular permeability and growth; stimulates angiogenesis</td>
<td>43</td>
</tr>
</tbody>
</table>

Tumor-*inhibiting* platelet proteins

| Location   | Name     | Function                                                        | Refs |
|------------|----------|                                                                |      |
| **Tumour-Inhibiting** |          |                                                                |      |
| α-granules | PF4      | Promotes blood coagulation; inhibits tumour growth and angiogenesis | 133  |
| TSP1       |          | Enhances platelet aggregation and inhibits angiogenesis         | 52   |
| VWF        |          | Matrix protein, forms multimers to aggregate platelets, binds GPIb-V-IX; inhibits tumour cell metastasis | 115  |
| PGK        |          | Glycolytic enzyme; anti-angiogenic factor                        | 163  |
| TIMP       |          | Inhibits matrix metalloproteinases, anti-angiogenic              | 106  |
| **Collagen α1(VIII) chain α1 chain α2 precursors (Endostatin)** |          | Angiogenesis inhibitor, derived from collagen type XVIII        | 43   |
| Dense granules | TFPI  | Serine protease inhibitor, inactivates TF-FXII coagulation       | 167  |
| PF4        |          | Promotes blood coagulation; inhibits tumour growth and angiogenesis | 133  |

Pro- and antiangiogenic regulators organize into separate, distinct α-granules in resting platelets.
PAR1 and PAR4 agonists differential release of VEGF and endostatin

Ma L et al. PNAS 2005;102:216-220
Platelet levels alter blood flow in ischemic limbs.

B

Before Ischemia  | Directly After  | 2d After  | 2wk After

PDS Control

PLT Infusion

IgG Control

PLT Depletion

Colored pixel

Blood Flow

0  250  500  750  1000
Platelet levels alter blood flow in ischemic limbs.

Angiogenesis requires platelet granules

Plasma alone (PL)

Room temp platelets (RT)

Cryopreserved platelets (FZ6)

Frozen platelets (FZ)

B

Blood Vessels/HPF

* *

Blood Vessels

0 10 20 30 40 50

PL FZ6 FZ RT

Platelet microparticles (PMP) induce angiogenesis in a dose dependent fashion
Elevated levels of angiogenic inhibitors in platelets of mice with dormant tumors

“In the ‘angiogenic’ tumors, the overall balance of angiogenesis may be tipped toward a more pro-angiogenic phenotype in a manner reflected in the platelet proteome.”

Lakka Klement et al. Blood 2009;113:2835-2842
Platelets in oncogenesis

Direct
- Immune evasion
- Tumor homing and adhesion to endothelium
- Differential release of pro- and anti-angiogenic factors from granules
- Increases vascular integrity / prevention of tumor hemorrhage

Indirect
- Endothelial cell migration
- Recruitment of bone marrow-derived cells
Platelets support angiogenesis while preventing excessive hemorrhage

Kisucka J et al. PNAS 2006;103:855-860
ADP-stimulated platelet releasate promotes angiogenesis.
Thromboxane-stimulated platelet releasate inhibits angiogenesis.
Platelet-endothelial interaction during tumor angiogenesis

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Platelets roll in tumor microvasculature

Platelets in oncogenesis

**Direct**
- Immune evasion
- Tumor homing and adhesion to endothelium
- Differential release of pro- and anti-angiogenic factors from granules
- Increases vascular integrity / prevention of tumor hemorrhage

**Indirect**
- Endothelial cell migration
- Recruitment of bone marrow-derived cells
Platelet releasate induces endothelial cell migration.
Platelet releasate induces endothelial cell migration.

Bovine aortic endothelial cells (25 x 10^3 in 100 μl)

Platelet releasate with or without appropriate inhibitors
Platelet α-granules are necessary for BMDC recruitment, tumor growth, and angiogenesis.

GPIIb/IIIa null ≈ Glanzmann
Dense granule null ≈ Hermansky-Pudlak
α-granule null ≈ gray platelet sx

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**In Summary...**

- Platelets contain both pro- and anti-angiogenic factors in separate α-granules
  - Different stimulants cause differential release of factors
  - Balance of pro- and anti-angiogenic agents are associated with rate tumor growth
  - Platelet microparticles induce angiogenesis
- Platelets increase tumor vascular integrity and prevent hemorrhage, independent of thrombus formation
- Platelets induce endothelial cell migration and bone marrow-derived cell homing to sites of angiogenesis

...respect the platelet
13 year old Male

• No bleeding complications during treatment
• Remains cancer free after 1+ years...
Food for thought...

• Platelet VEGF or TSP-1 levels as diagnostic tool for tumor recurrence?
• Platelet antagonists as cancer therapy?
• Platelet transfusion practices in oncology?
Some angiogenic proteins made, others taken up

Matrigel 100 µL
(~50 ng $^{125}$I VEGF)

Counts per gram of tissue ($\times 10^5$)

Liver  | Matrigel  | Spleen  | Kidney  | Plasma  | Platelets

©2009 by American Society of Hematology
Megakaryocytes *produce* Thrombospondin-1 in presence of tumor