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Research Institute of MUHC
Department of Pediatrics

Single cell coagulomes as constituents of the oncogene-driven coagulant phenotype in brain tumours

Janusz Rak

9th International Conference on Thrombosis and Hemostasis Issues in Cancer (ICTHIC)-Bergamo– April 15th , 2018

Disclosures for Janusz Rak

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Speakers Bureau	*[No relevant conflicts of interest to declare*
Other (Specify)	*[No relevant conflicts of interest to declare*

Thank You

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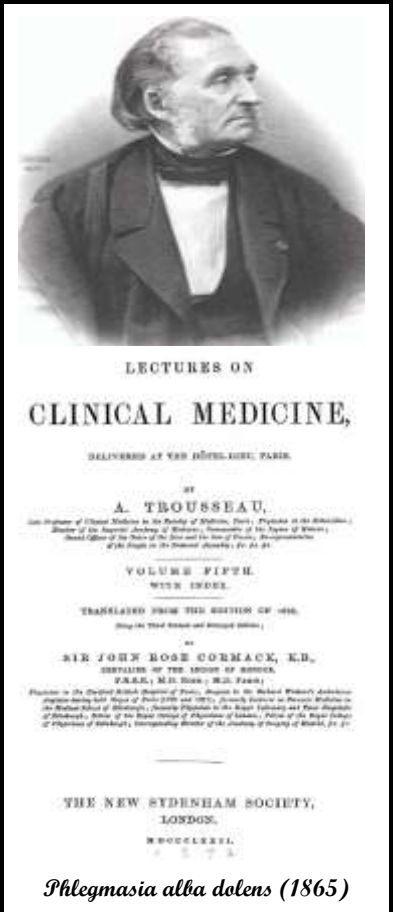
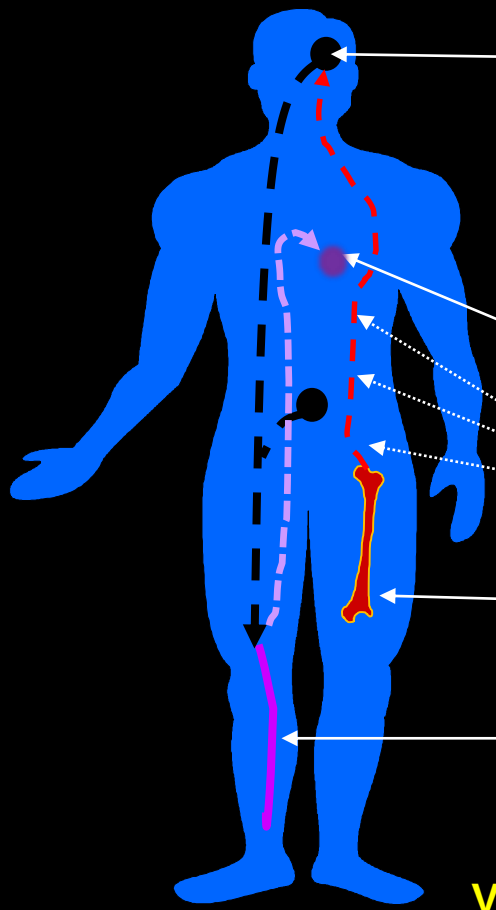
Complex and systemic nature of cancer - the *vascular fulcrum*



Phlegmasia alba dolens (1865)

Thrombosis associated with occult cancer - *Trousseau syndrome*

Vascular system is central to both intrinsic and iatrogenic triggers of systemic cancer progression



Tumor
tissue damage
angiogenesis
inflammation
metastasis
shedding
therapy
stasis

Secondary vascular impact (PE)

Cytokines?
BMDCs?
EVs/MPs?

Bone marrow

Peripheral vascular impact (DVT)

Venous thromboembolism (VTE)

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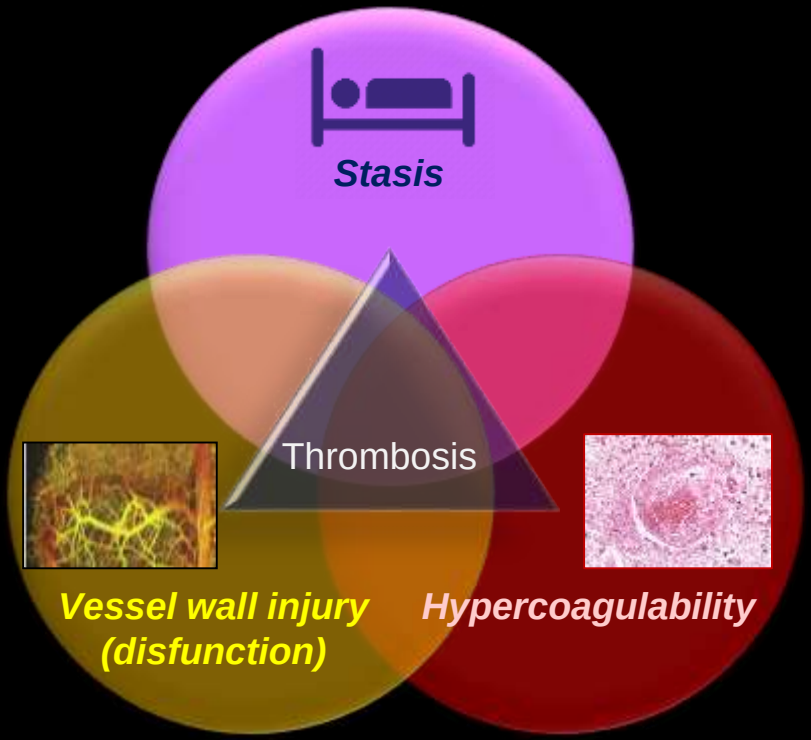
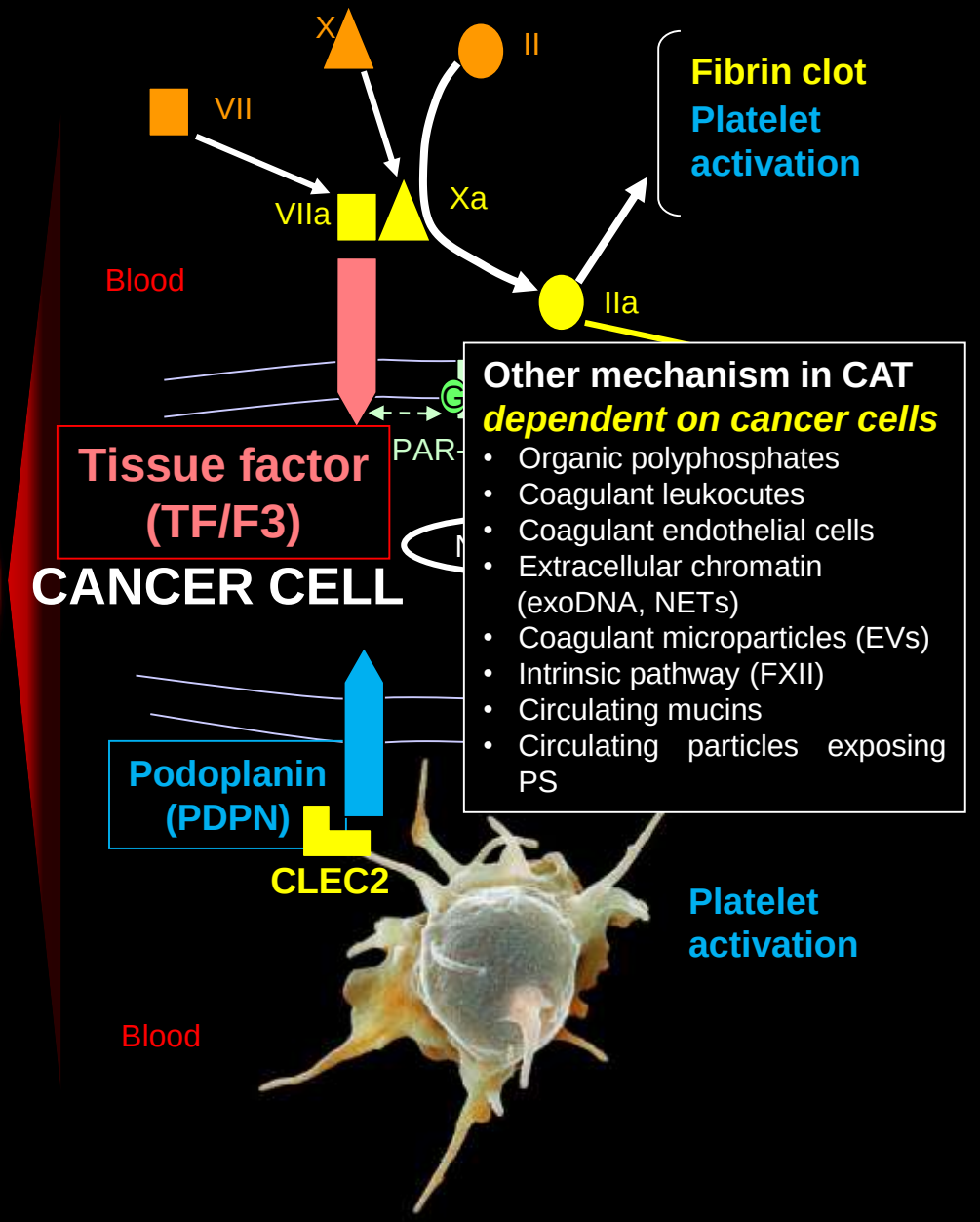
Pathomechanisms of cancer associated thrombosis (CAT)

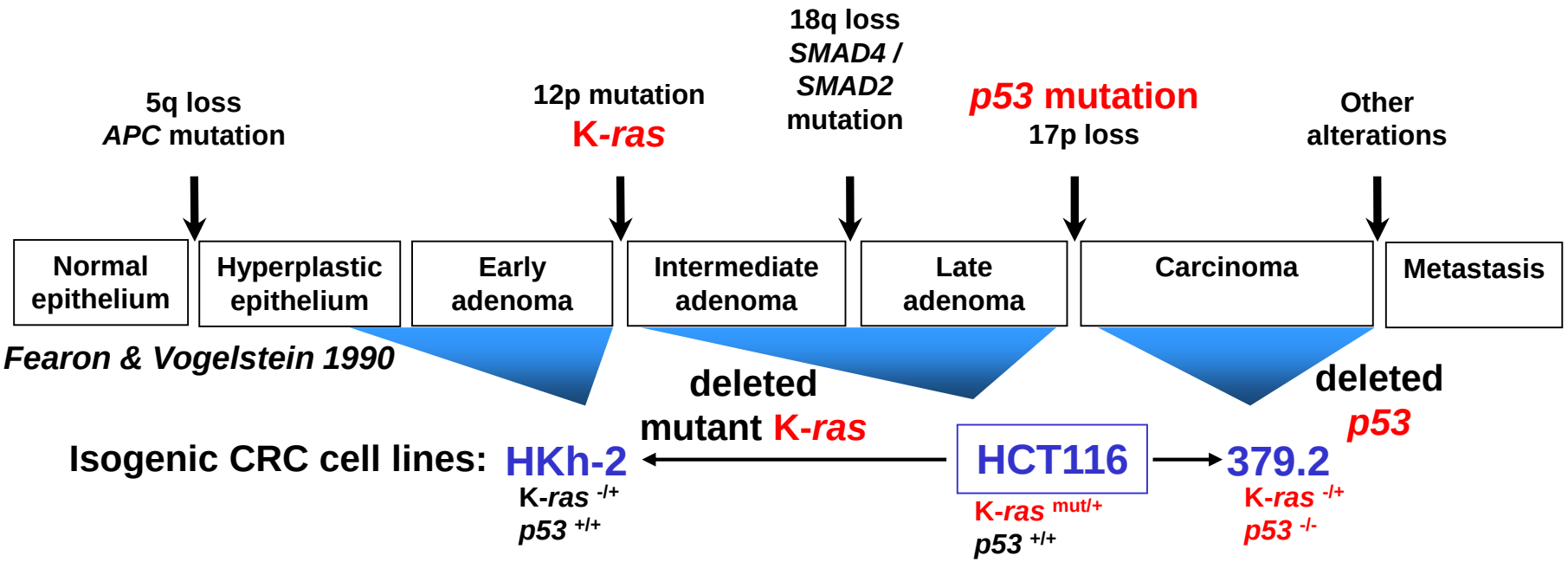


Virchow's Triad



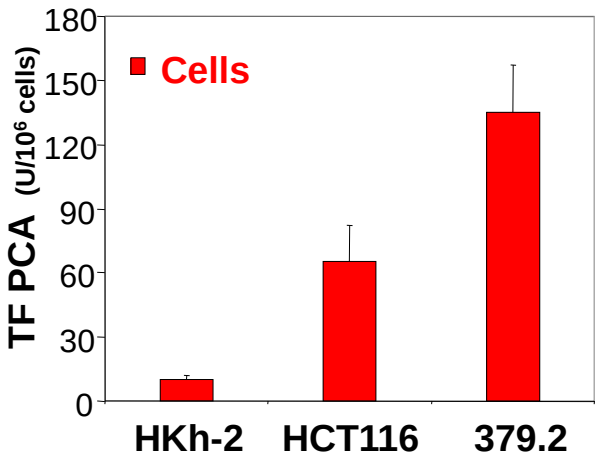
Is CAT a function of cancer genes?

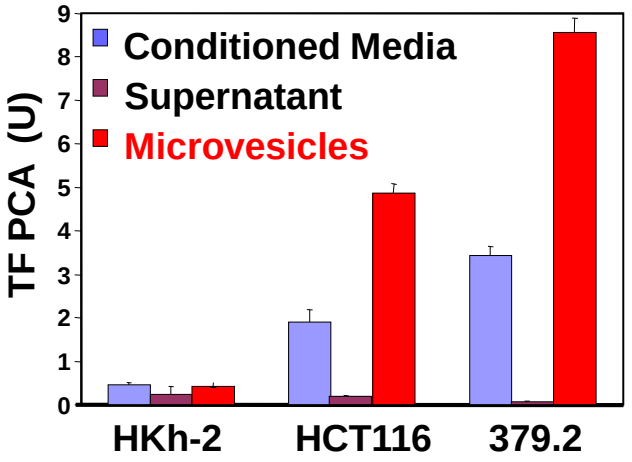
Tissue factor and genetic progression of colorectal cancer



Yu (Rak) et al Blood 2005

Oncogene-driven TF Procoagulant Activity in Cancer





Journal of Thrombosis and Haemostasis, **13**: 998–1003 **2015** DOI: 10.1111/jth.12910

ORIGINAL ARTICLE

Tumor oncogene (*KRAS*) status and risk of venous thrombosis in patients with metastatic colorectal cancer

S. ADES,* S. KUMAR,* M. ALAM,† A. GOODWIN,‡ D. WECKSTEIN,§ M. DUGAN,¶ T. ASHIKAGA,** M. EVANS,† C. VERSCHRAEGEN* and C. E. HOLMES*

**Department of Medicine, Vermont Cancer Center, University of Vermont, Burlington, VT; †The James, Ohio State University Wexner Medical Center, Columbus, OH; ‡Department of Pathology and Laboratory Medicine, University of Vermont, Burlington, VT; §New Hampshire Oncology-Hematology PA, Hookset, NH; ¶New England Cancer Specialists, Scarborough, ME; and **Department of Math and Statistics, University of Vermont, Burlington, VT, USA*

Table 2 Incidence of DVT and VTE among patients with mutated and WT *KRAS*

	All patients (n = 172) (%)	Patients with mutated *KRAS* (n = 65) (%)	Patients with WT* *KRAS* (n = 107) (%)	Odds ratio† (95% CI)
DVT‡	26 (15.1)	15 (23.1)	11 (9.4)	2.62 (1.12–6.12)
PE§	18 (10.5)	8 (12.3)	10 (9.3)	1.36 (0.51–3.65)
VTE¶	40 (23.3)	21 (32.3)	19 (17.8)	2.21 (1.08–4.53)

*Wild-type. †Logistic regression analysis. ‡Deep venous thrombosis. §Pulmonary embolism. ¶Venous thromboembolism.

Journal of Thrombosis and Haemostasis, **16**: 293–302 **2018** DOI: 10.1111/jth.13926

ORIGINAL ARTICLE

Genes associated with venous thromboembolism in colorectal cancer patients

9 matched pt pairs – 30 differentially expressed genes

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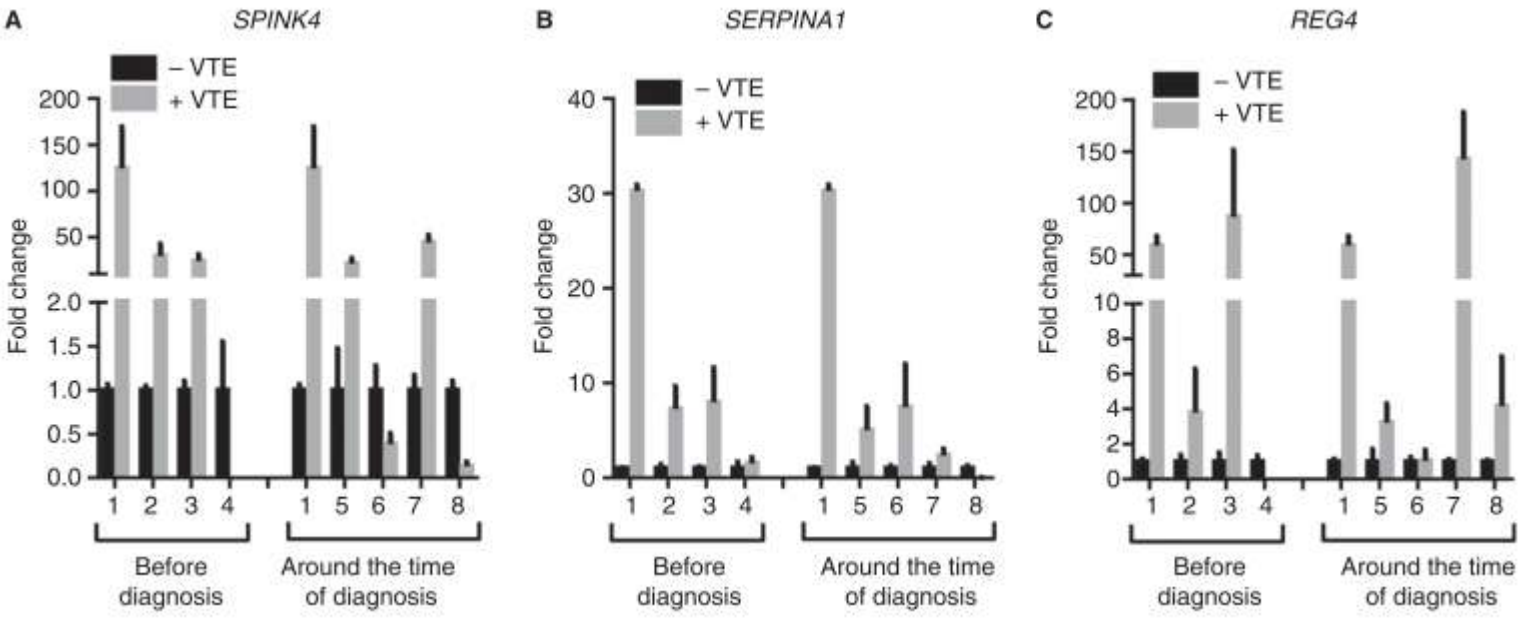
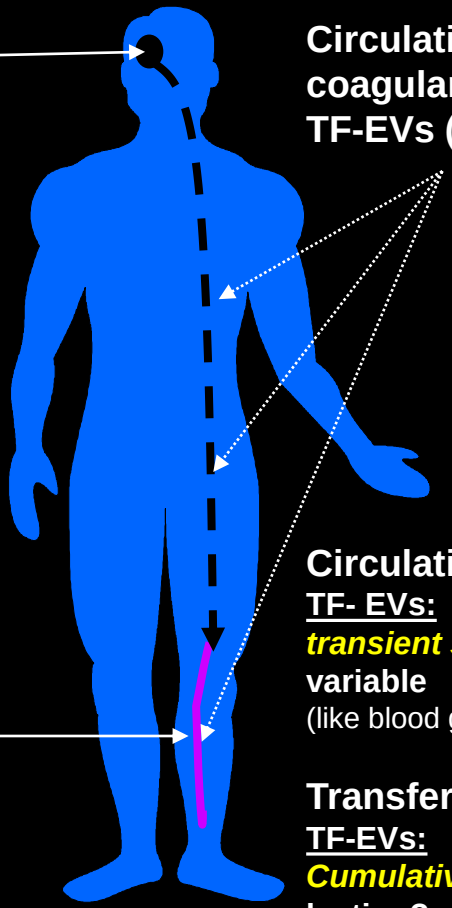
KRAS target



***Emerging - Sussman/Khorana** – Diff. gene expression in cancers with and without VTE ASH 2017*

Extracelluar vesicles (EVs) and *intercellular communication* in CAT

Tumor
mutations
gene expression
tissue damage
angiogenesis
inflammation
stasis

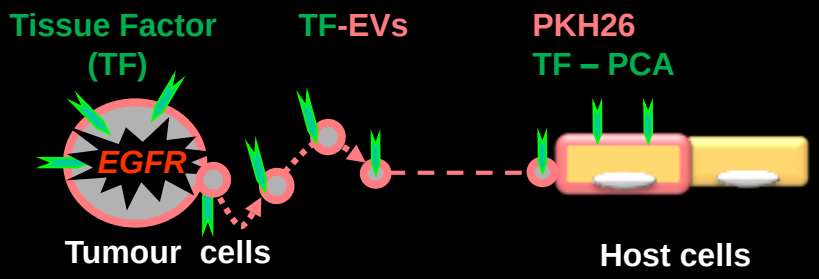


Peripheral vascular impact (DVT)

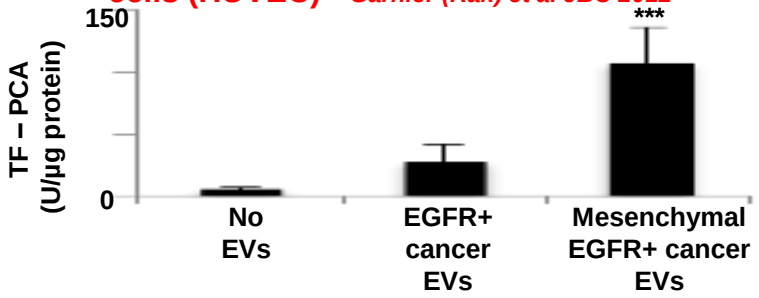
Circulating coagulant TF-EVs (MPs)?

Circulating
TF- EVs:
transient state
variable
(like blood glucose)

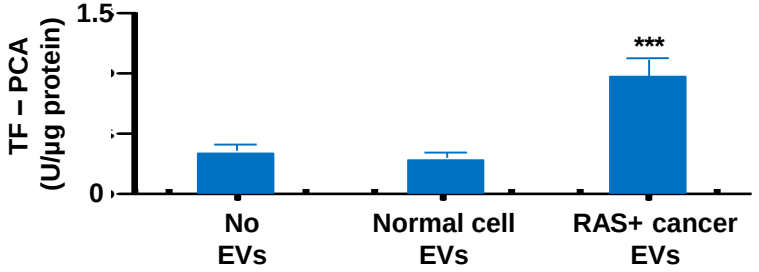
Transferred
TF-EVs:
Cumulative state
lasting?
(like A1C Hb)



Transfer of TF-procoagulant activity to endothelial cells (HUVEC) – *Garnier (Rak) et al JBC 2012*



Induction of TF-procoagulant activity in myeloid cells (HL60) – *Chennakrishaiah (Rak) 2018 – in revision*



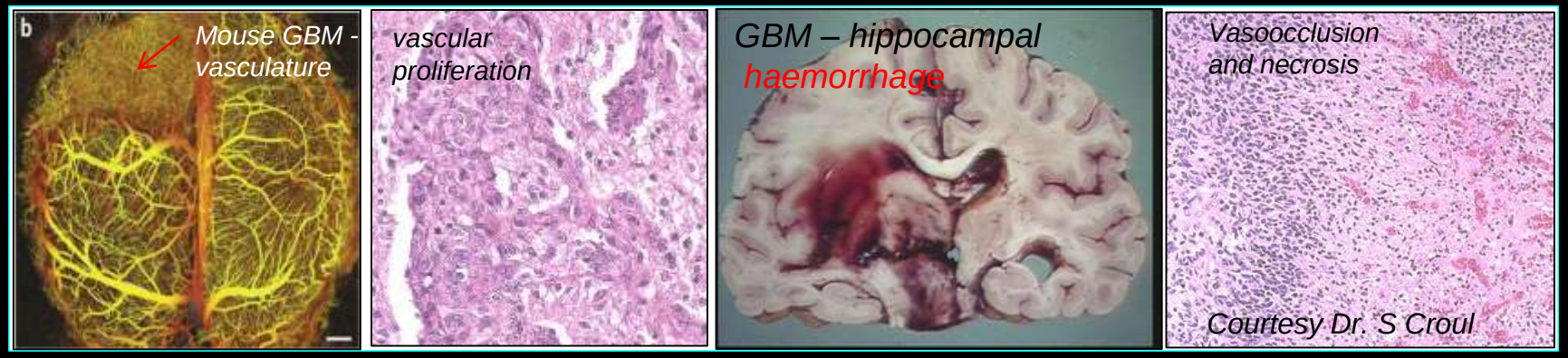
CAT in brain tumours and their subtypes

Tumour types differ with respect to thrombotic risks (and mechanisms

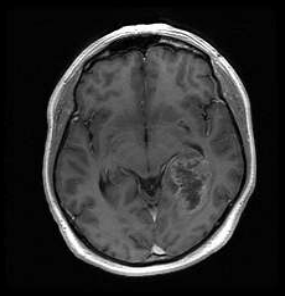
Cancer Type *(Stein et al 2006, suppl by Timp 2013)*	**VTE rate/pt-years** *(Wun & Whyte 2009)*	**% TE**
Pancreas	14.0%	4.68
Brain	**11.1%**	**3.89**
Colon	2.7%	2.02
Leukemia	7.4% (AML); 3.1 (ALL)	1.81
Breast	0.9%	1.74
Bladder	1.7%	1.09
Oral	ND	0

Distinct nature and risk of *VTE in glioblastoma*

(Perry et al 2012; Unruh et al 2016; LeRhun & Perry 2016)



Glioblastoma multiforme (GBM) – the epitome of a medical calamity

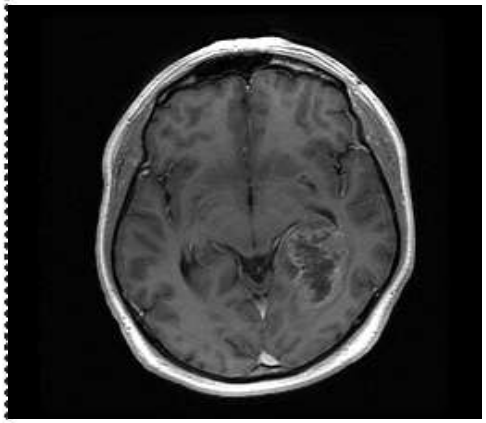


- The most frequent astrocytic primary brain tumour in adults
- Mostly (95%) insidious development without (known) precursor lesions
- Highly vascular and necrotic(diagnostic)
- Onset at 61 – 64 years of age, incidence - 3/100,00/year
- *Median overall survival ~ 15 months*, almost uniformly fatal (3-5% - 5 y), severe morbidity
- Molecularly studied to a significant depth but to now major clinical consequence
- Therapy – Stupp protocol (2005) – gross total resection>chemoradiation (TMZ)
- Second line bevacizumab – anti-edema effect with no significant impact on survival
- *Targeted therapies – multiple tried and failed*
- Immunotherapy – trials ongoing early failures already recorded (CheckMate -143 Phase 3 trial (NCT02017717) with Opdivo/Nivolumab)

Diversity of driver mutations and *oncogenic pathways* in GBM subtypes

causative factors, diagnostic markers and therapeutic targets

Glial progenitor cells

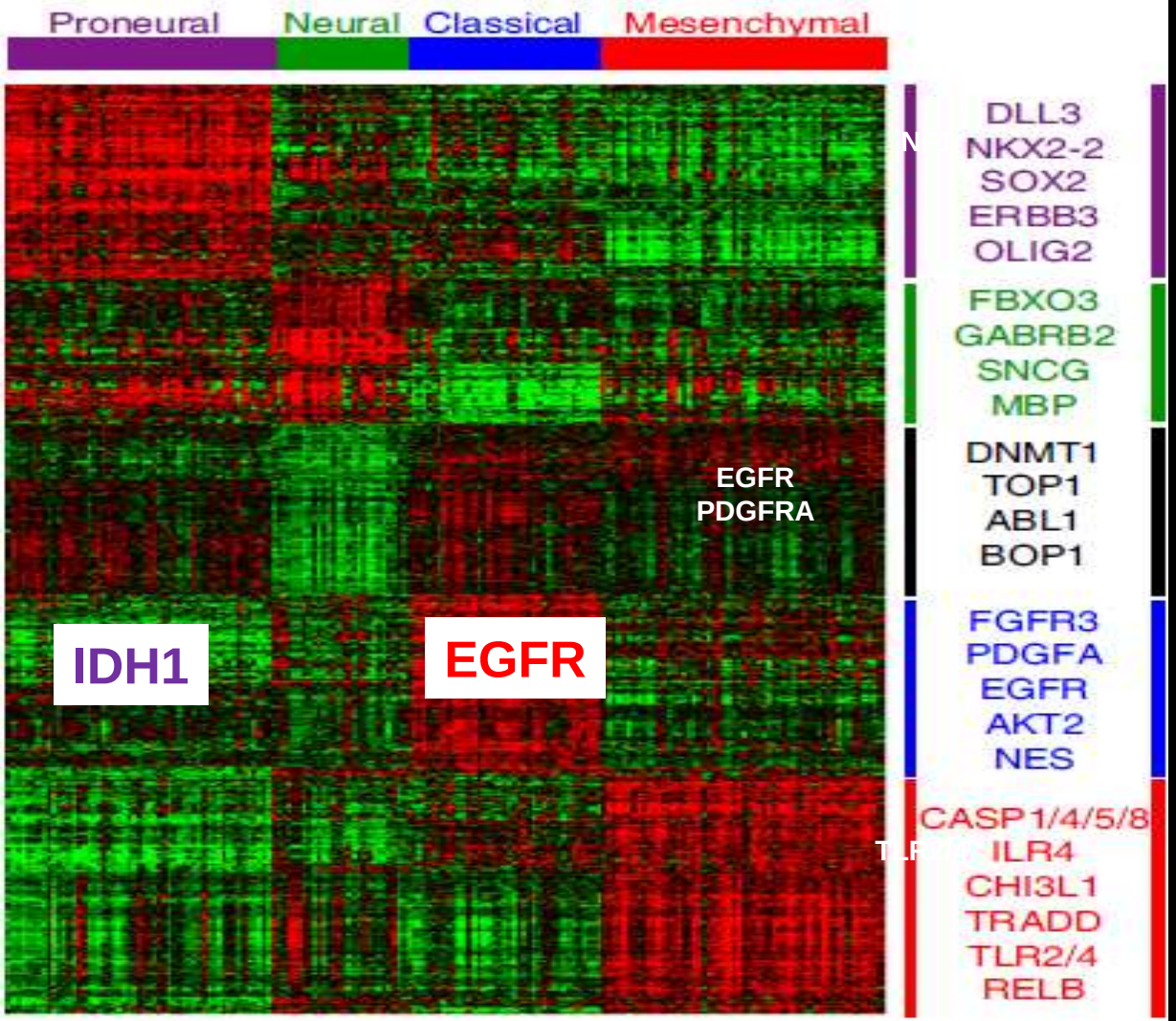


EGFRvIII mutation (~20%)

EGFR amplification (~35%)
TP53 mutation (~30%)
PTEN mutation (~25%)
NF1 alteration (~20%)
LOH 10p (~70%)
LOH 10q (~70%)

Primary glioblastoma

TCGA - Verhaak et al 2010/2011



Adapted from Ohgashi & Kleihues 2011; Sturm/Jabado et al 2012, Verhaak et al 2010

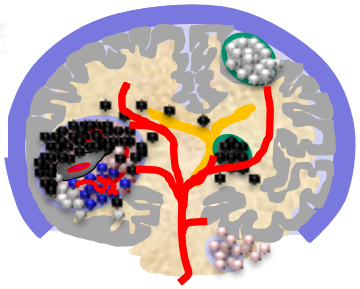
Molecular subtype-related *coagulomes in glioblastoma* (GBM)

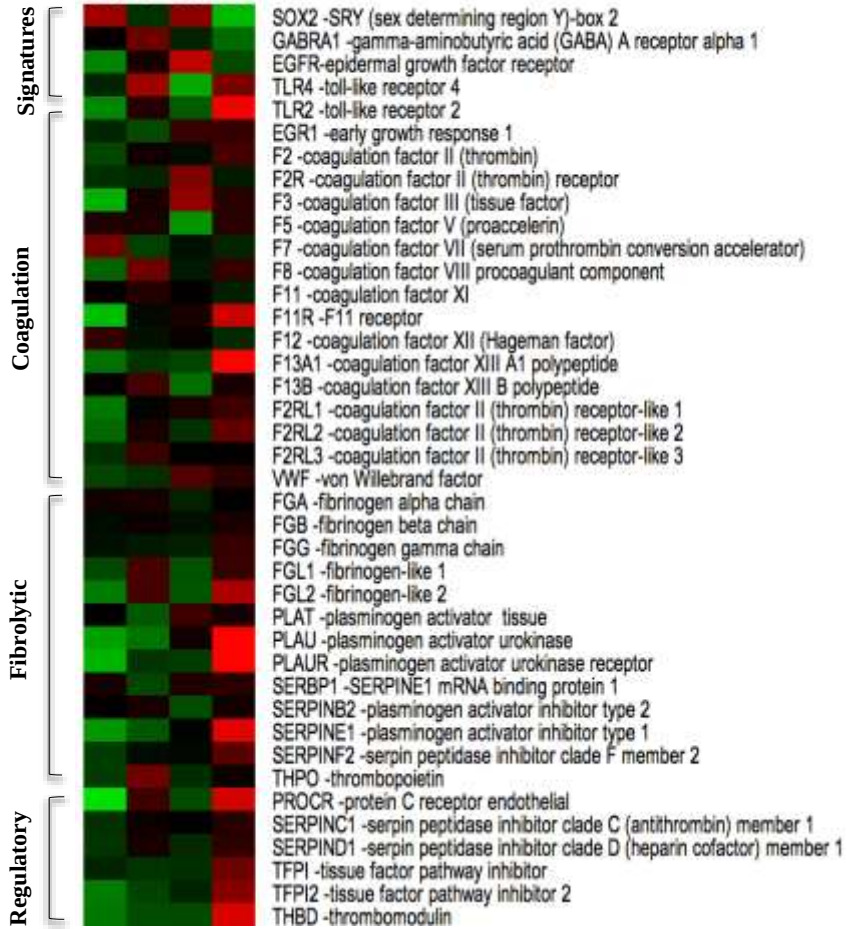
GBM coagulomes

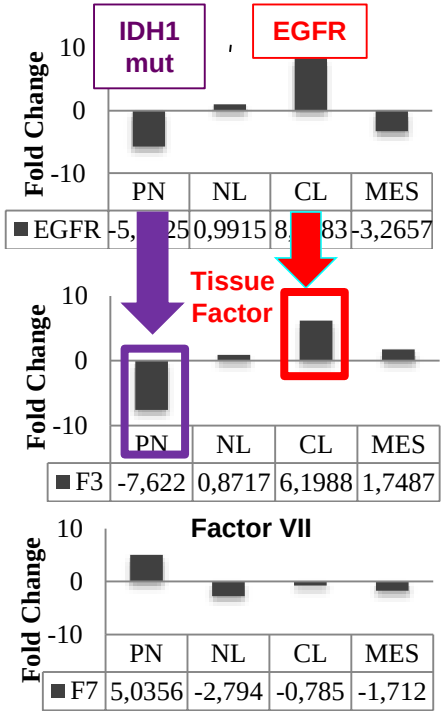
Coagulation-related gene expression profile in glioblastoma is defined by molecular disease subtype

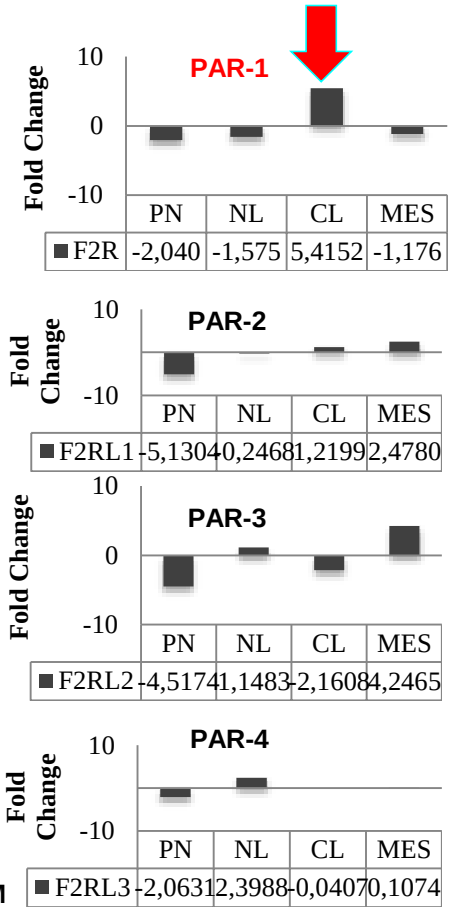
N. MAGNUS, N. GERGES, N. JABADO and J. RAK
Montreal Children's Hospital Research Institute, McGill University, Montreal, QC, Canada

J Thromb Haemost 2013; **11**: 1197–200.









PN – Proneural GBM
NL – Neural GBM
CL – Classical GBM
MES – Mesenchymal GBM

Adapted from Magnus *et al (Rak)* – *JTH 2013*

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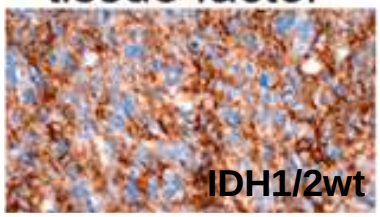
Mutant IDH1 defines low thrombosis risk in proneural GBM subtype

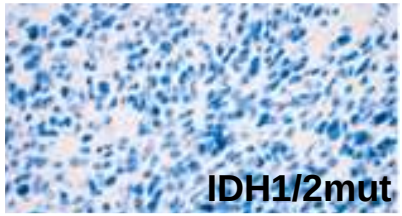
Acta Neuropathol (2016) 132:917–930
DOI 10.1007/s00401-016-1620-7

ORIGINAL PAPER

Mutant IDH1 and thrombosis in gliomas

tissue factor





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Why would anyone anticoagulate IDH1 mutant GBM patients?

Table 1 Patient characteristics for the discovery and validation cohorts, stratified by *IDH1/2*

Characteristic	No. of patients	Discovery Cohort ($N = 169$)		
		IDH1/2 Wild-type,	*IDH1/2* Mutant, no. (%)	*P*
Intratumoral microthrombi				
Yes	206	100 (85.5)	1 (1.9)	<0.001
No	111	17 (14.5)	51 (98.1)	
VTE present	61	30 (25.6)	0 (0.0)	<0.001
VTE absent	237	87 (74.4)	45 (100.0)	

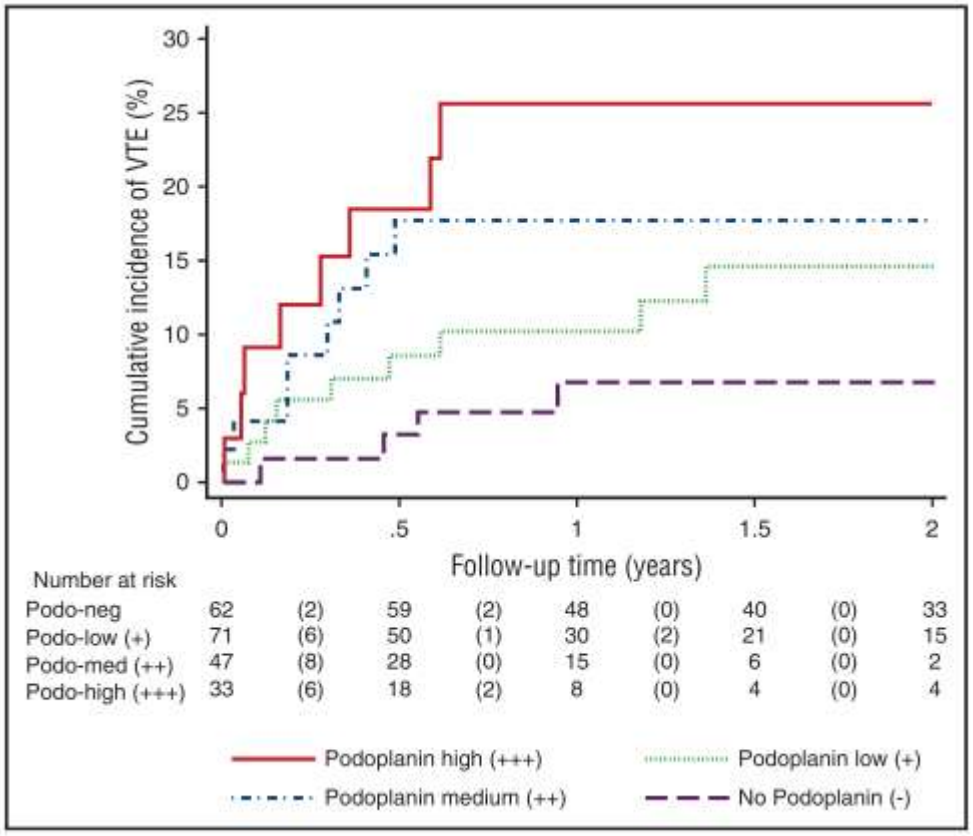
Podoplanin levels predict VTE in and is *down-regulated in proneural GBM* along with TF

blood

BLOOD, 30 MARCH 2017 • VOLUME 129, NUMBER 13

Podoplanin expression in primary brain tumors induces platelet aggregation and increases risk of venous thromboembolism

Julia Riedl,[1,2] Matthias Preusser,[2,3] Pegah Mir Seyed Nazari,[1,2] Florian Posch,[1,2,4] Simon Panzer,[5] Christine Marosi,[2,3] Peter Birner,[6] Johannes Thaler,[1,2] Christine Brostjan,[7] Daniela Lötsch,[2,8] Walter Berger,[2,8] Johannes A. Hainfellner,[9] Ingrid Pabinger,[1,2] and Cihan Ay[1,2,10]

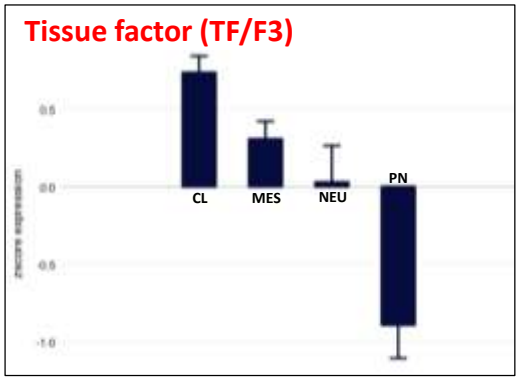


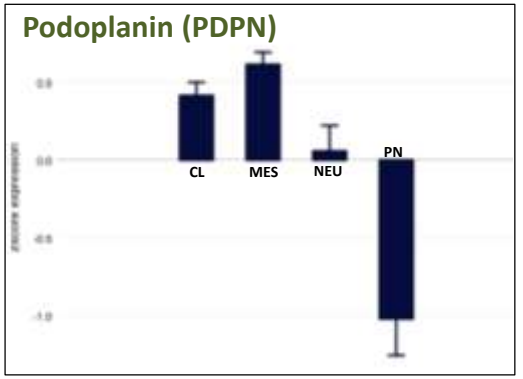
Thrombosis Research

Full Length Article

Single cell coagulomes as constituents of the oncogene-driven coagulant phenotype in brain tumours

Nadim Tawil, Shilpa Chennakrishnaiah, Rayhaan Bassawon, Radia Johnson, Esterina D'Asti, Janusz Rak





PNAS

Tissue factor expression provokes escape from tumor dormancy and leads to genomic alterations

Nathalie Magnus[a], Delphine Garnier[a], Brian Meehan[a], Serge McGraw[a], Tae Hoon Lee[a], Maxime Caron[b], Guillaume Bourque[b], Chloe Milsom[c], Nada Jabado[a], Jacquetta Trasler[a], Rafal Pawlinski[d], Nigel Mackman[d], and Janusz Rak[a,1]

3544–3549 | PNAS | March 4, 2014 | vol. 111 | no. 9

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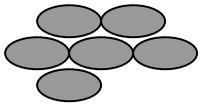
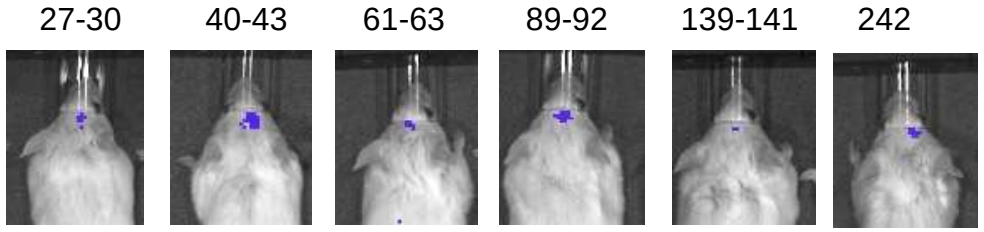
Could clotting system change the biology of GBM?

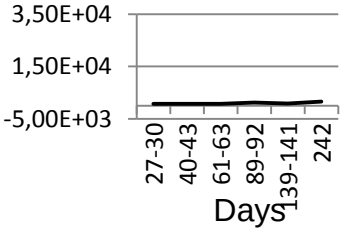
U373



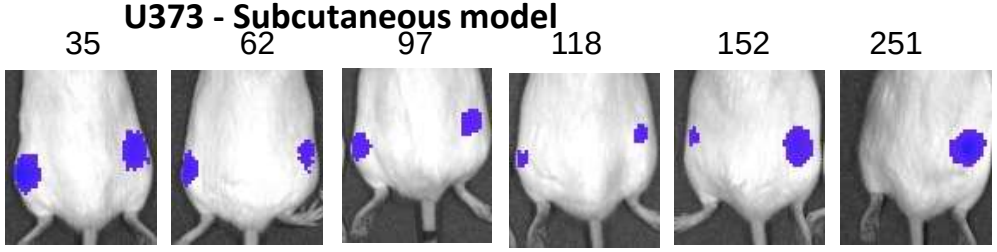
No TF
Non tumorigenic
Non Angiogenic

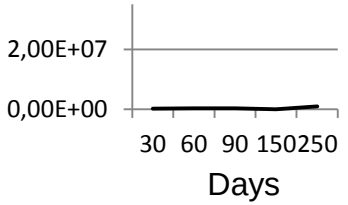
U373 - Intracranial (orthotopic model of dormancy)





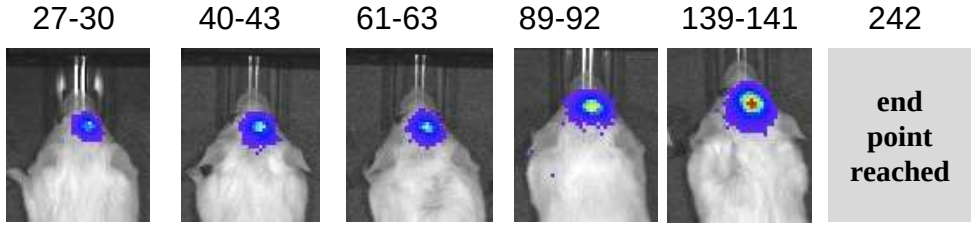
U373 - Subcutaneous model

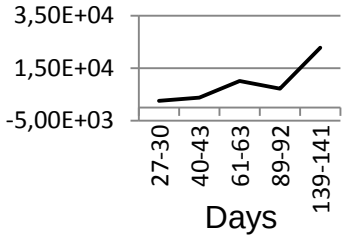




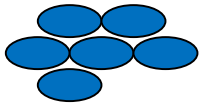
hTF expression

TF U373 G11- Intracranial (orthotopic model)



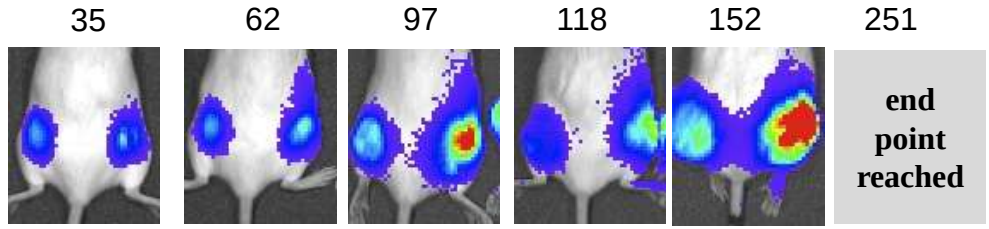


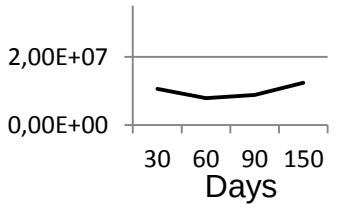
TF U373



HIGH TF
Procoagulant

TF U373 G11 - Subcutaneous model



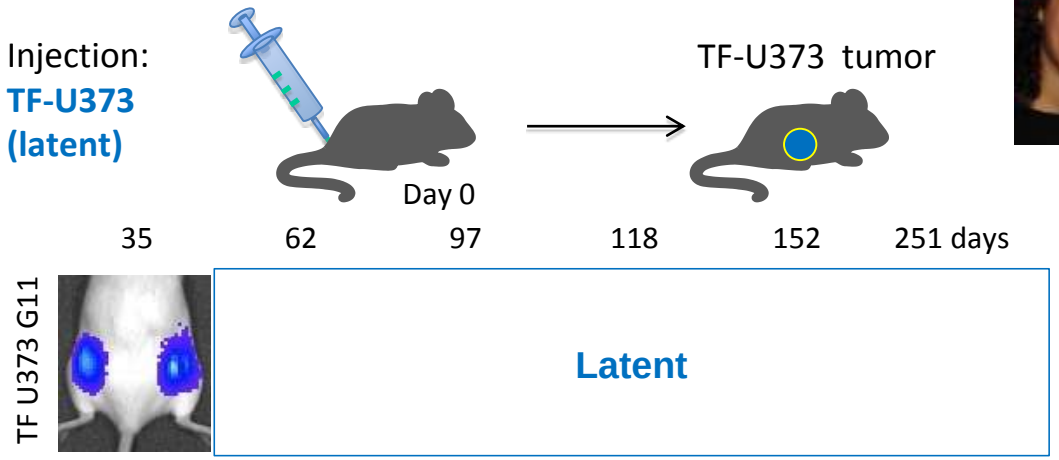


Procoagulant microenvironment may change *cellular properties* of GBM

Coagulant microenvironment disrupts tumour dormancy

- Non-coagulant **U373** human glioma cells cells don't form tumours in mice
- Tissue factor expressing (coagulant) **TF-U373** cells form tumours after prolonged latency

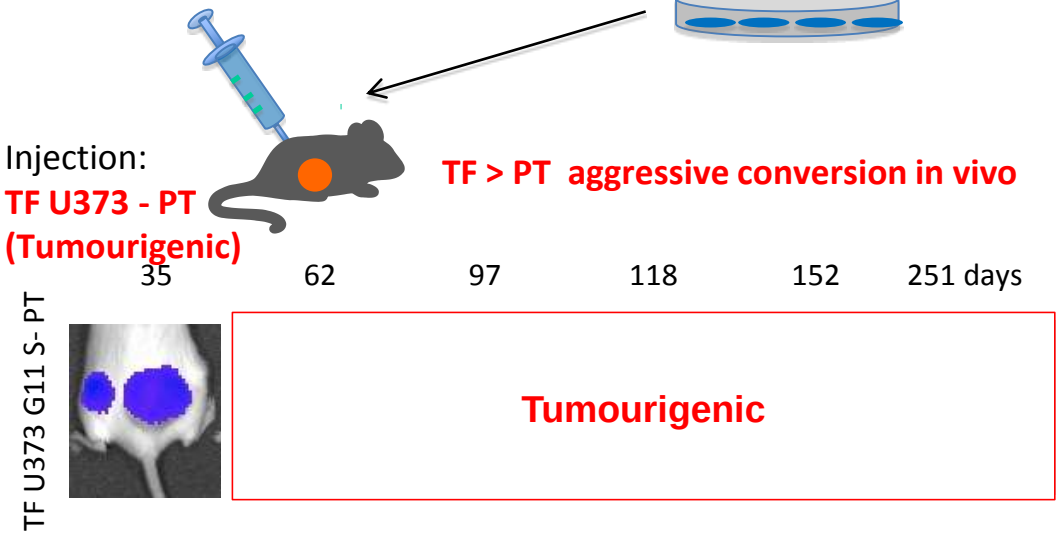
Nathalie Magnus



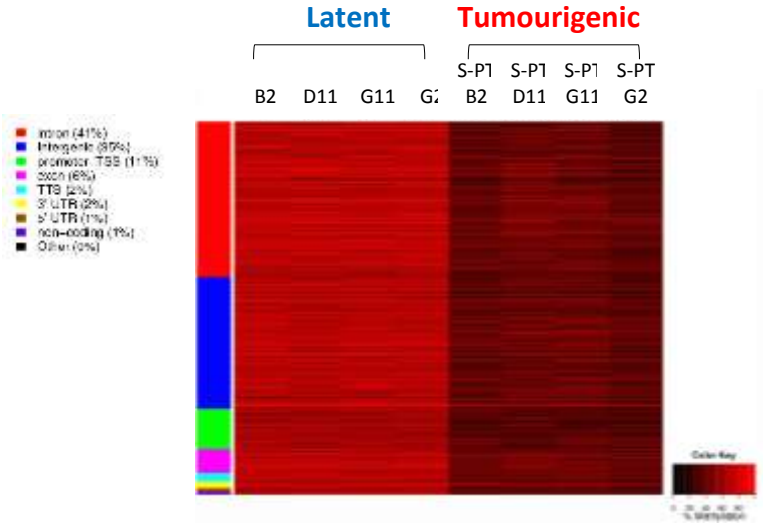
Coagulant microenvironment **may permanently alter the cancer cell:**

- ***phenotype*** **(aggressiveness)**
- ***gene expression*** **(transcriptome)**
- ***genome*** **(CNV)**
- ***epigenome*** **(methylome)**

coagulation > angiogenesis > Inflammation



Epigenome (DNA methylation – RRBS)



Magnus (Rak) et al PNAS. 2014

Pharmacological *targeting* of tumour-related *tissue factor* alters the expression of *microRNA* by cancer cells *in vivo*

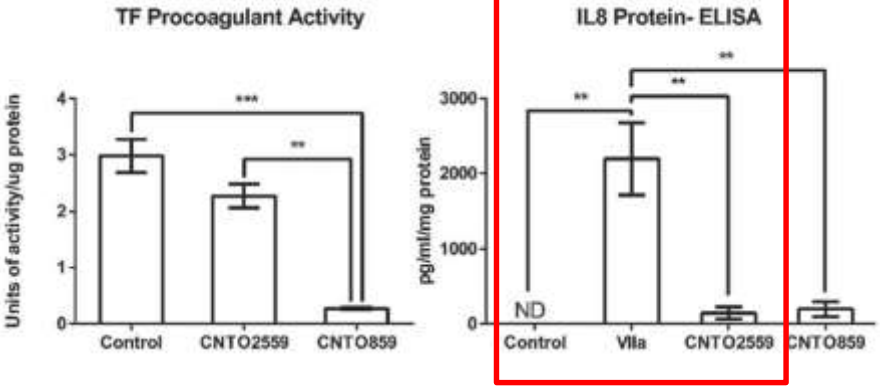
Inhibition of tissue factor signaling in breast tumour xenografts induces widespread changes in the microRNA expression profile

Esterina D'Asti [a], G. Mark Anderson [b], Janusz Rak [a,*]

[a] McGill University, Research Institute of the McGill University Health Centre, Montreal Childrens' Hospital, Montreal, Quebec, Canada
[b] Centocor, Inc., Radnor, PA, USA

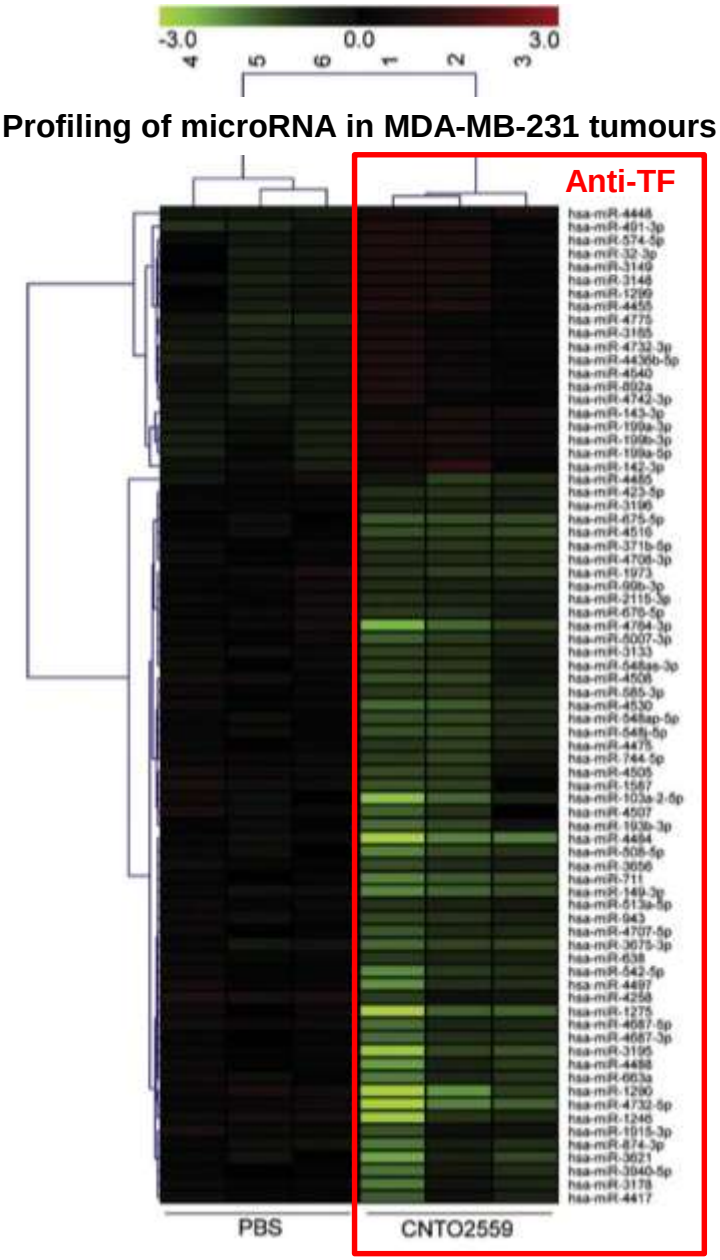
Biochemical and Biophysical Research Communications 494 (2017) 700–705

Anti-TF signalling

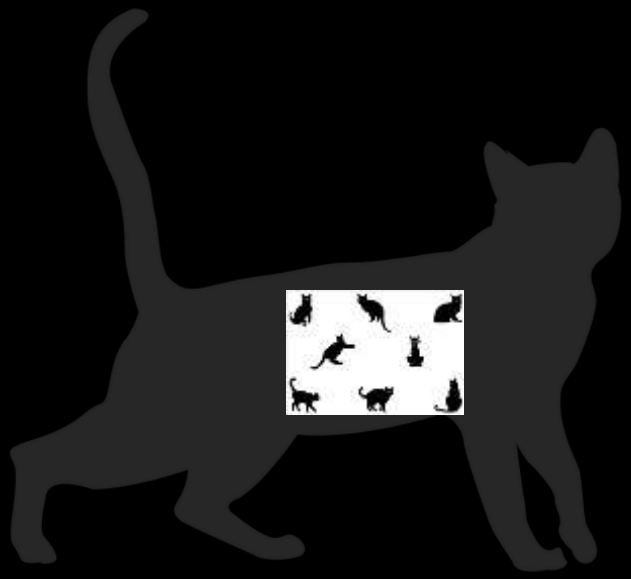


Obliteration human TF ***signaling and not coagulant*** properties of cancer cells (MDA-MB-231) by a specific function blocking anti-TF antibody changes the expression of 75 microRNAs in vivo.

Profiling of microRNA in MDA-MB-231 tumours

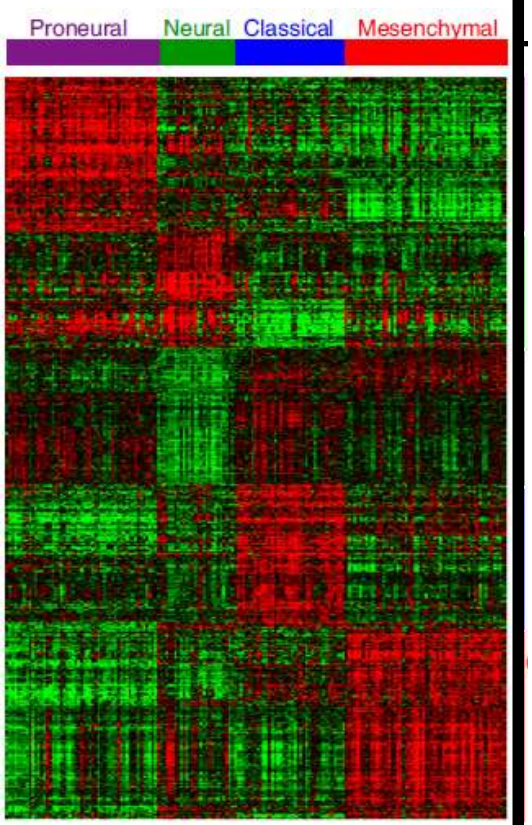


How many CATs in a CAT?

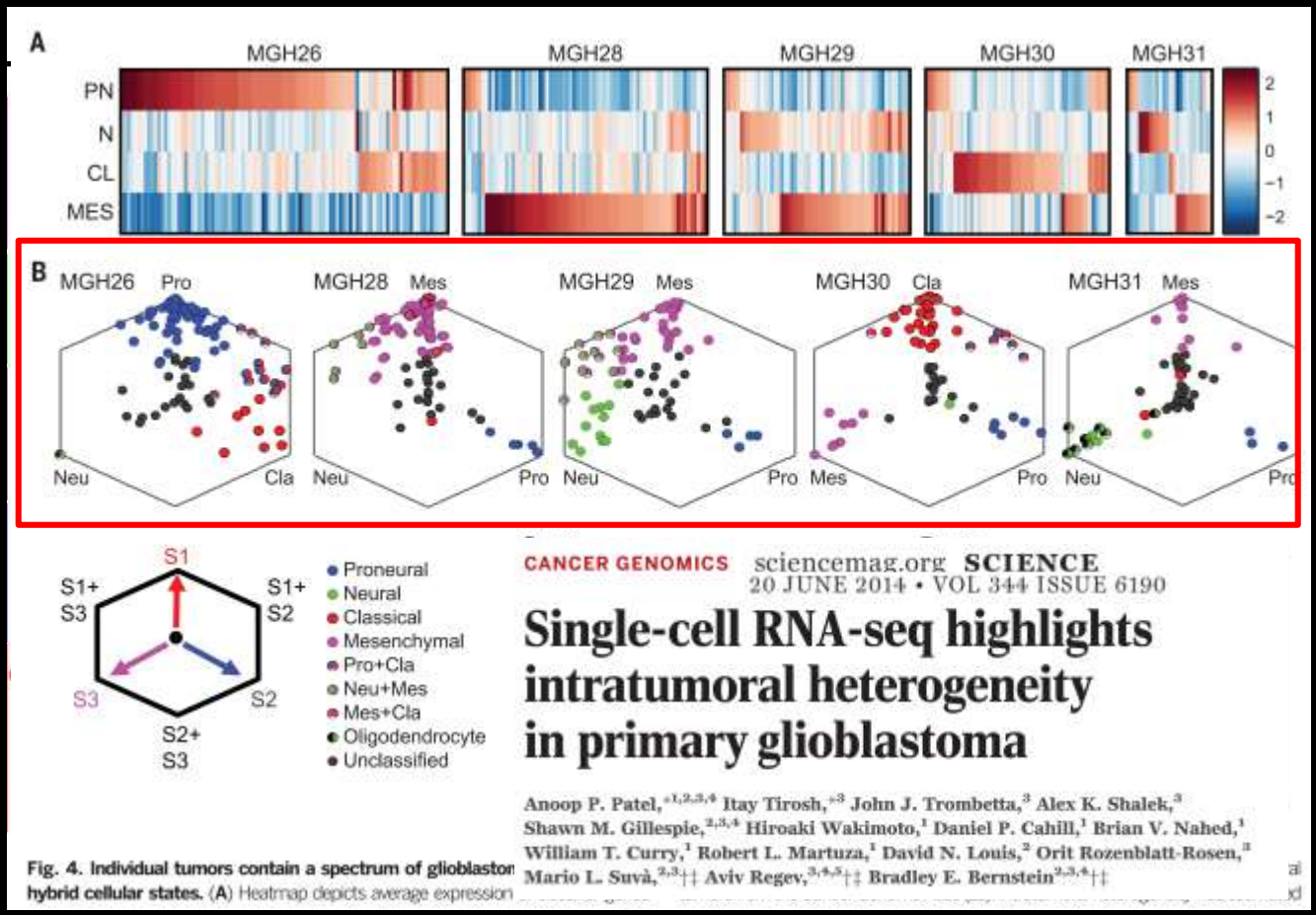


Janusz.rak@mcgill.ca

Cellular heterogeneity in GBM revealed through *single cell sequencing*



TCGA - Verhaak et al 2010



CANCER GENOMICS sciencemag.org SCIENCE
20 JUNE 2014 • VOL 344 ISSUE 6190

Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma

Anoop P. Patel,[*1,2,3,4] Itay Tirosh,[*3] John J. Trombetta,[3] Alex K. Shalek,[3] Shawn M. Gillespie,[2,3,4] Hiroaki Wakimoto,[1] Daniel P. Cahill,[1] Brian V. Nahed,[1] William T. Curry,[1] Robert L. Martuza,[1] David N. Louis,[2] Orit Rozenblatt-Rosen,[3] Mario L. Suvà,[2,3]†‡ Aviv Regev,[3,4,5]†‡ Bradley E. Bernstein[2,3,4]†‡

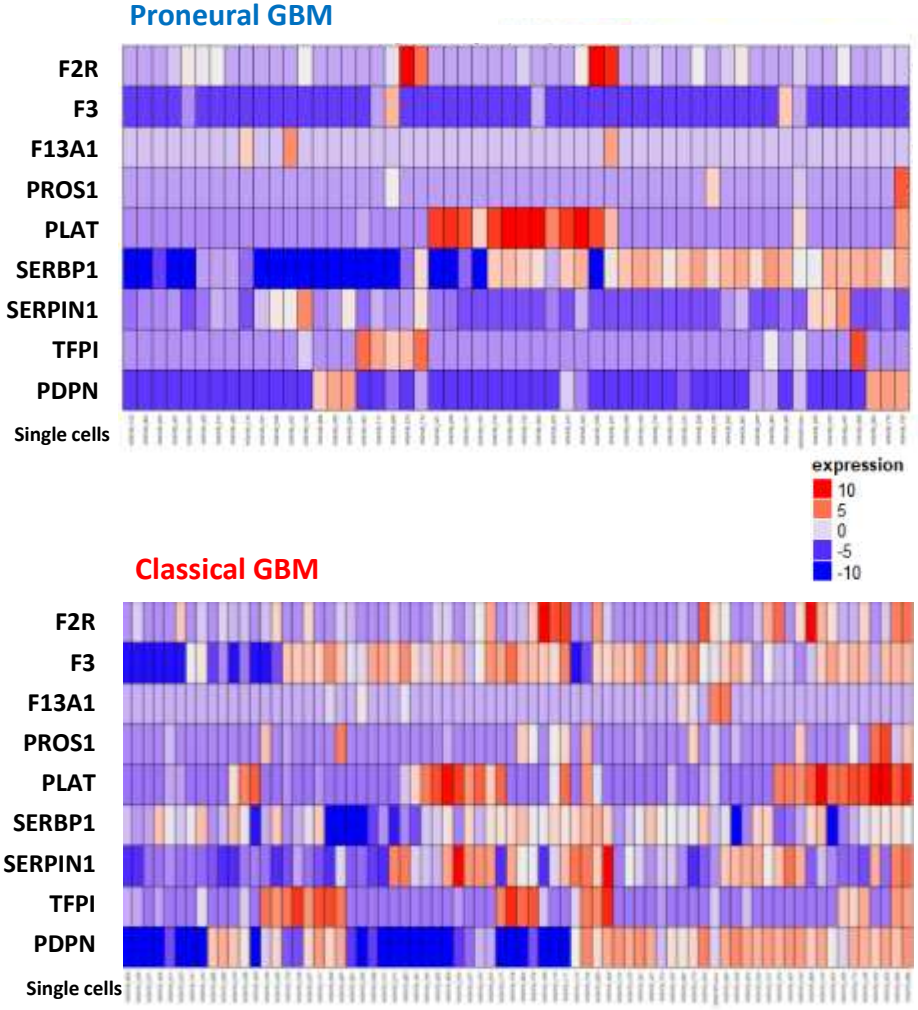
Fig. 4. Individual tumors contain a spectrum of glioblastom hybrid cellular states. (A) Heatmap depicts average expression

If GBM as a cellular mosaic, is *coagulome also a mosaic?*

Cellular heterogeneity in GBM – *combinatorial coagulome*

Tawil (Rak) et al Thrombosis Res. 2018

Single Cell Coagulome in Human Glioblastoma

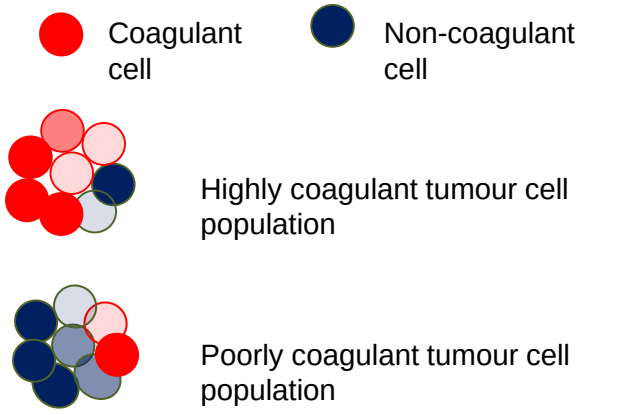


Full Length Article

Single cell coagulomes as constituents of the oncogene-driven coagulant phenotype in brain tumours

Nadim Tawil, Shilpa Chennakrishnaiah, Rayhaan Bassawon, Radia Johnson, Esterina D'Asti, Janusz Rak

Combinatorial coagulant phenotype in cancer



Some emerging questions:

- **Are mechanisms of coagulation system activation unspecfic or cancer-specific - *thrombosis or thromboses* ?**
- **Could cancer *genome and epigenome* (oncogene and oncomir profiles) be risk factors for cancer associated thrombosis (CAT)?**
- **Could VTE and *anticoagulants change the biology of cancer (how)*?**
- **Should cancer patients be molecularly *stratified* for subtype-specific and *personalized management of CAT ?***
- **What is the role of tumour cell hetereogeneity in CAT? Are cancer coagulomes *composits of heterogeneous single cell coagulomes?***

Disfunctional lab 'family' in Montreal

Thank you