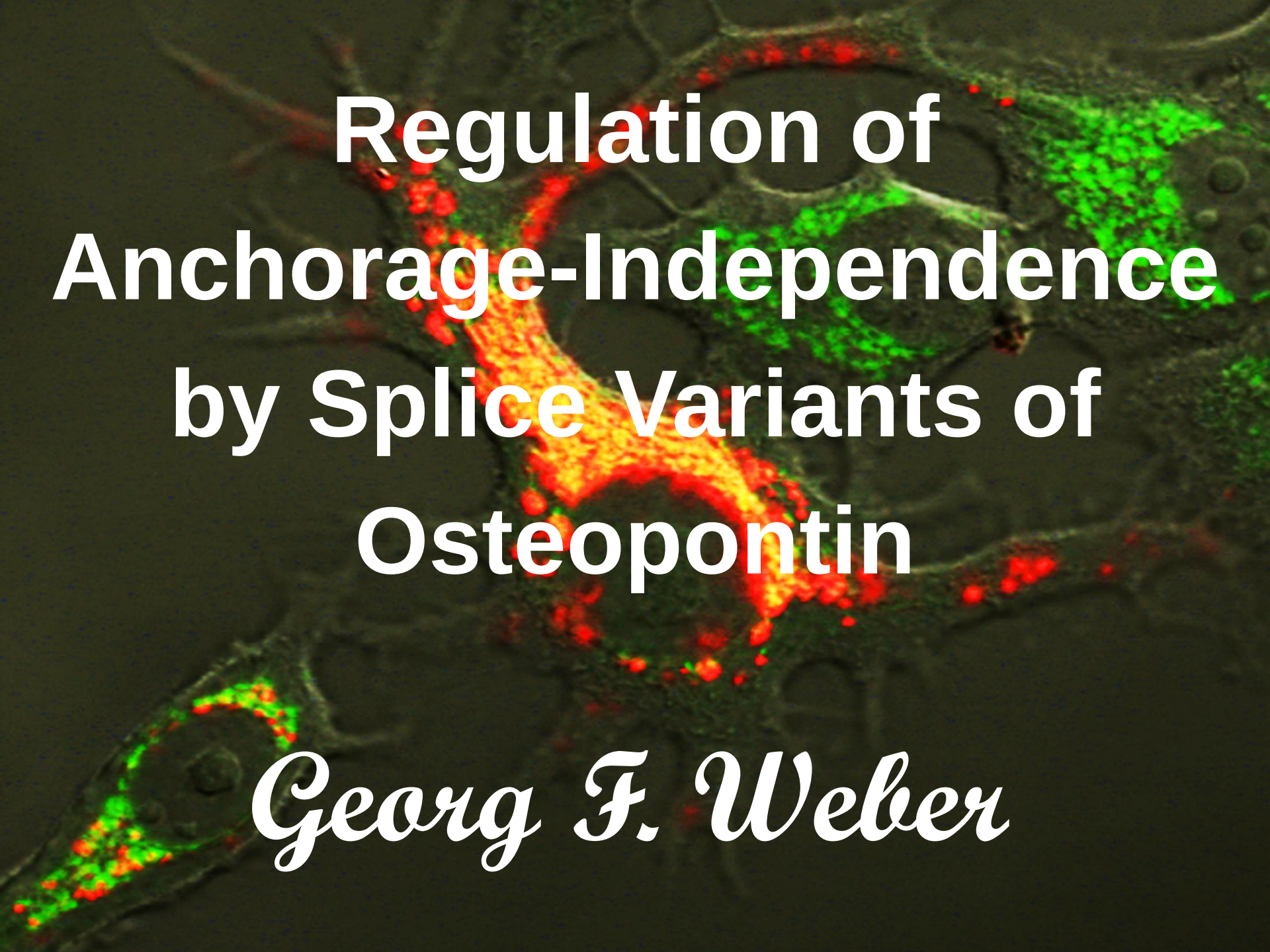
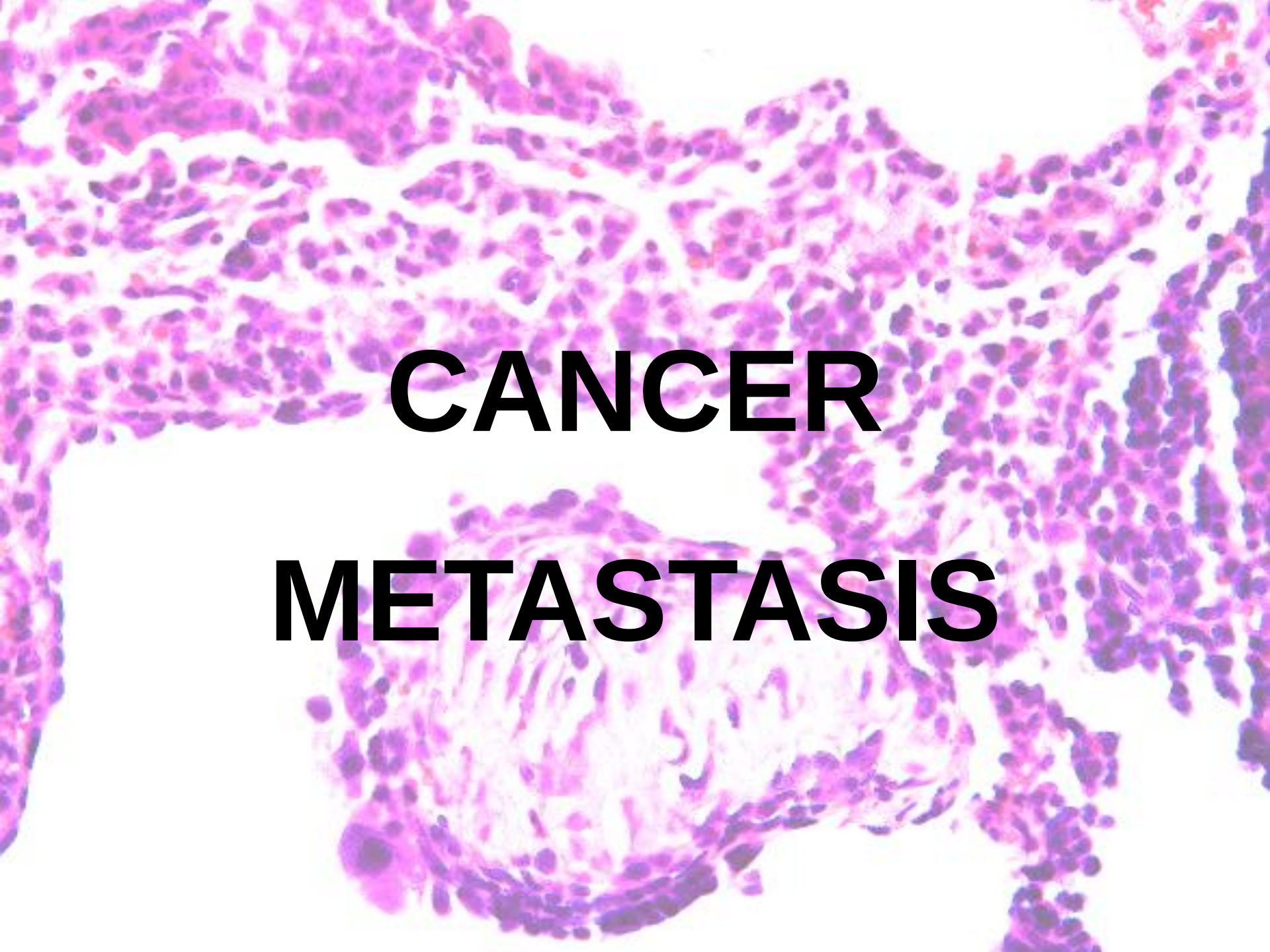
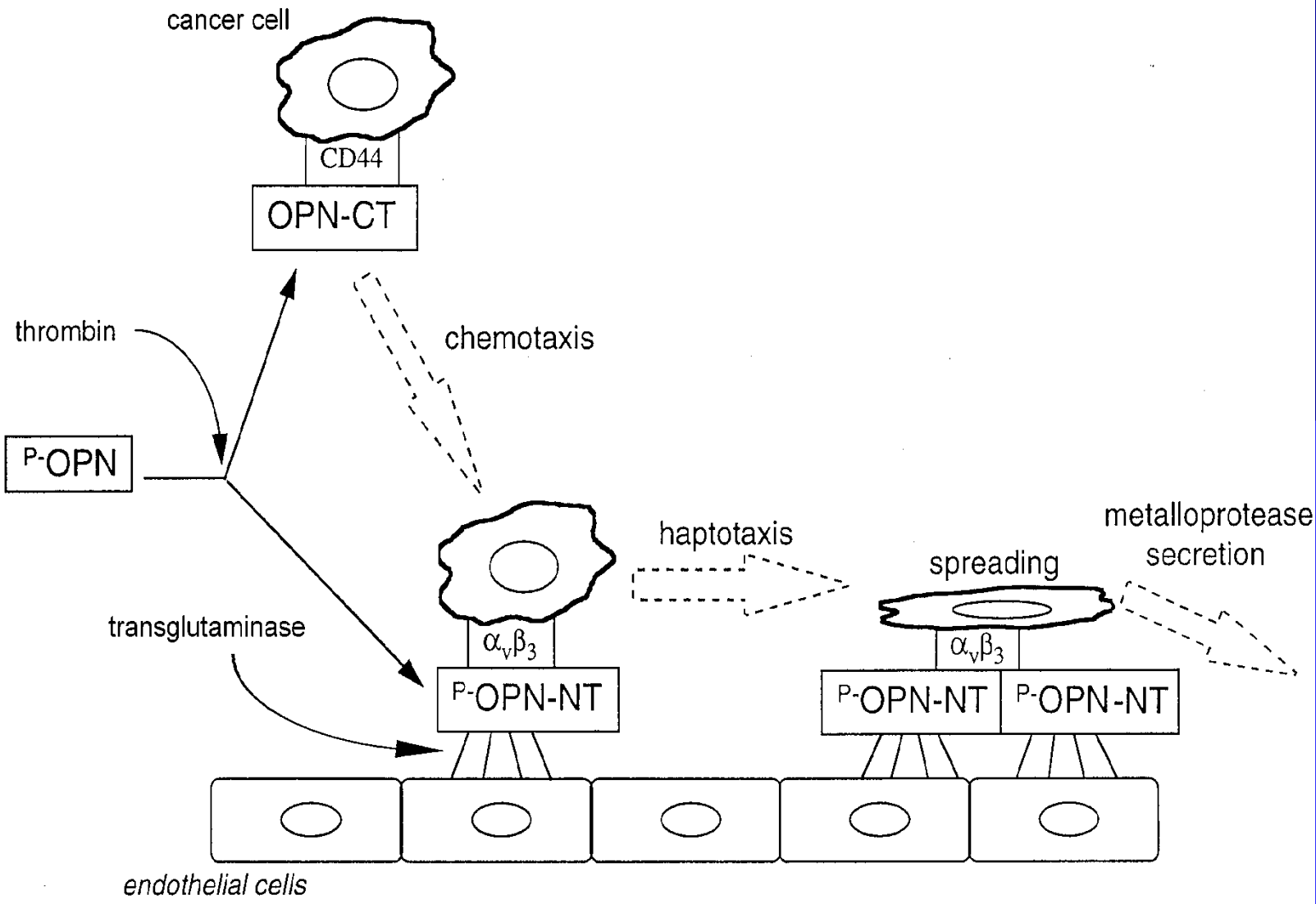

Regulation of
Anchorage-Independence
by Splice Variants of
Osteopontin
Georg F. Weber

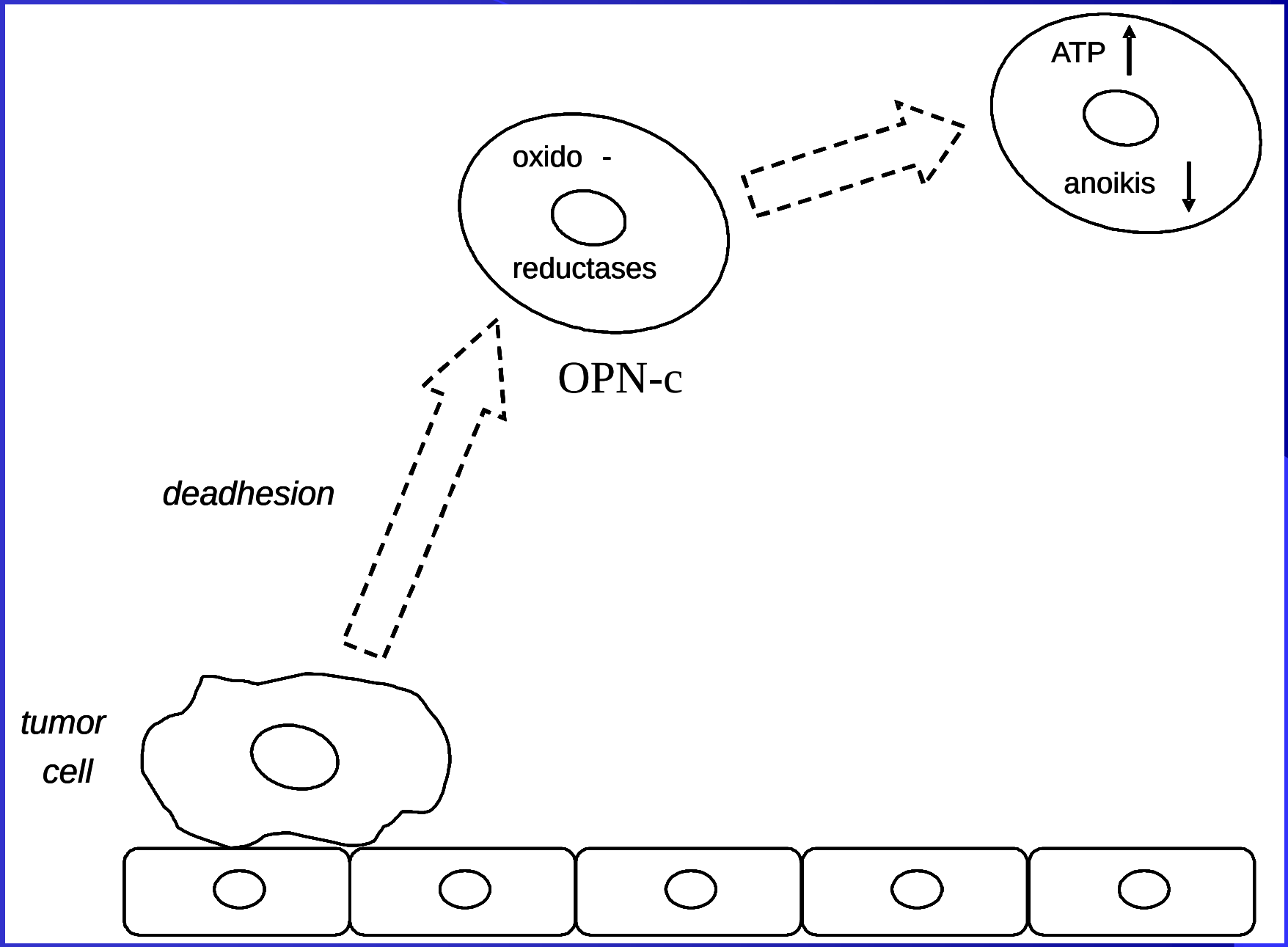

CANCER
METASTASIS

OSTEOPONTIN IN CANCER INVASION



Weber Cancer Letters, 2008; 270: 181-190

OSTEOPONTIN IN ANCHORAGE INDEPENDENCE

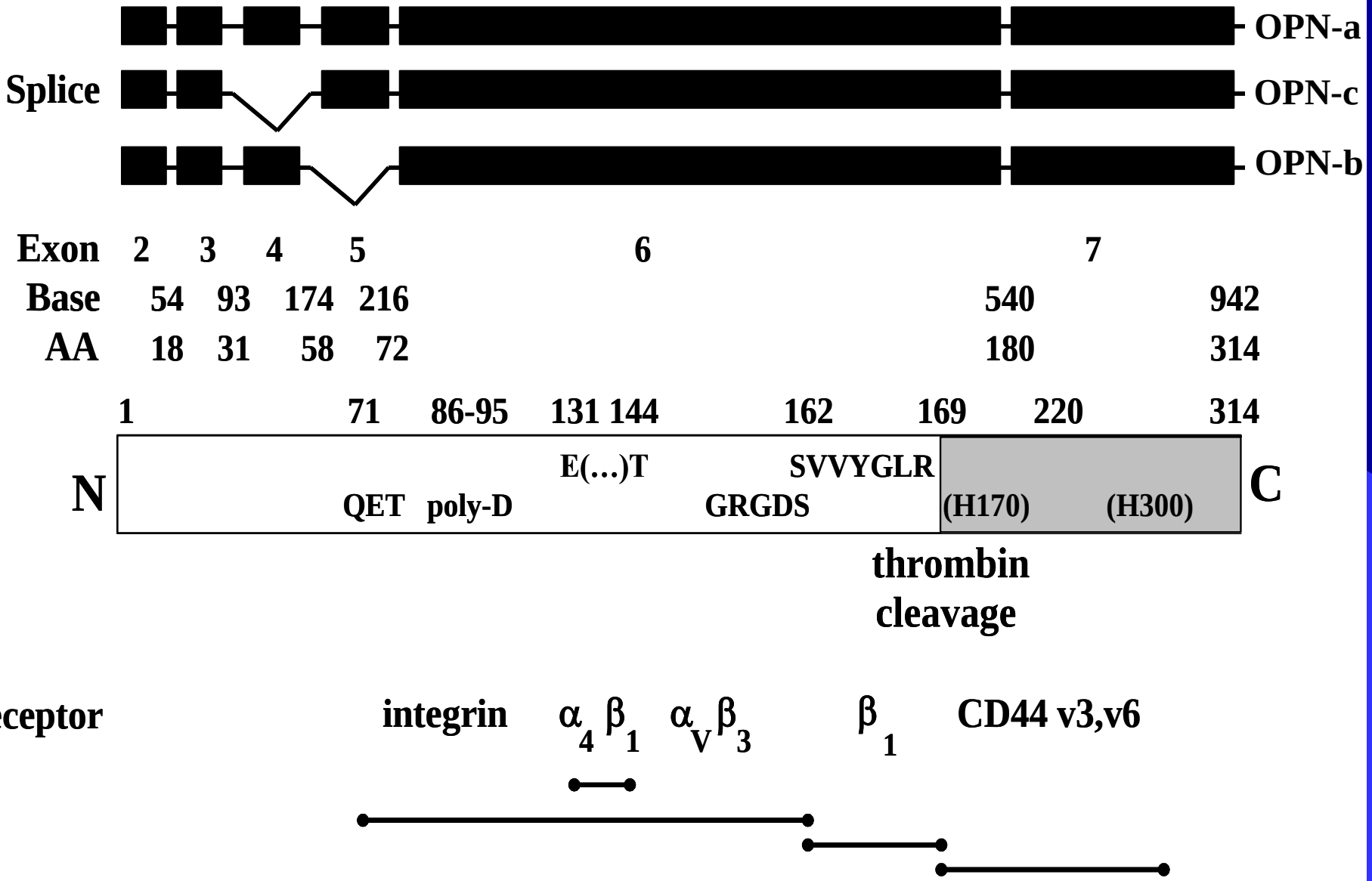


ANCHORAGE INDEPENDENCE

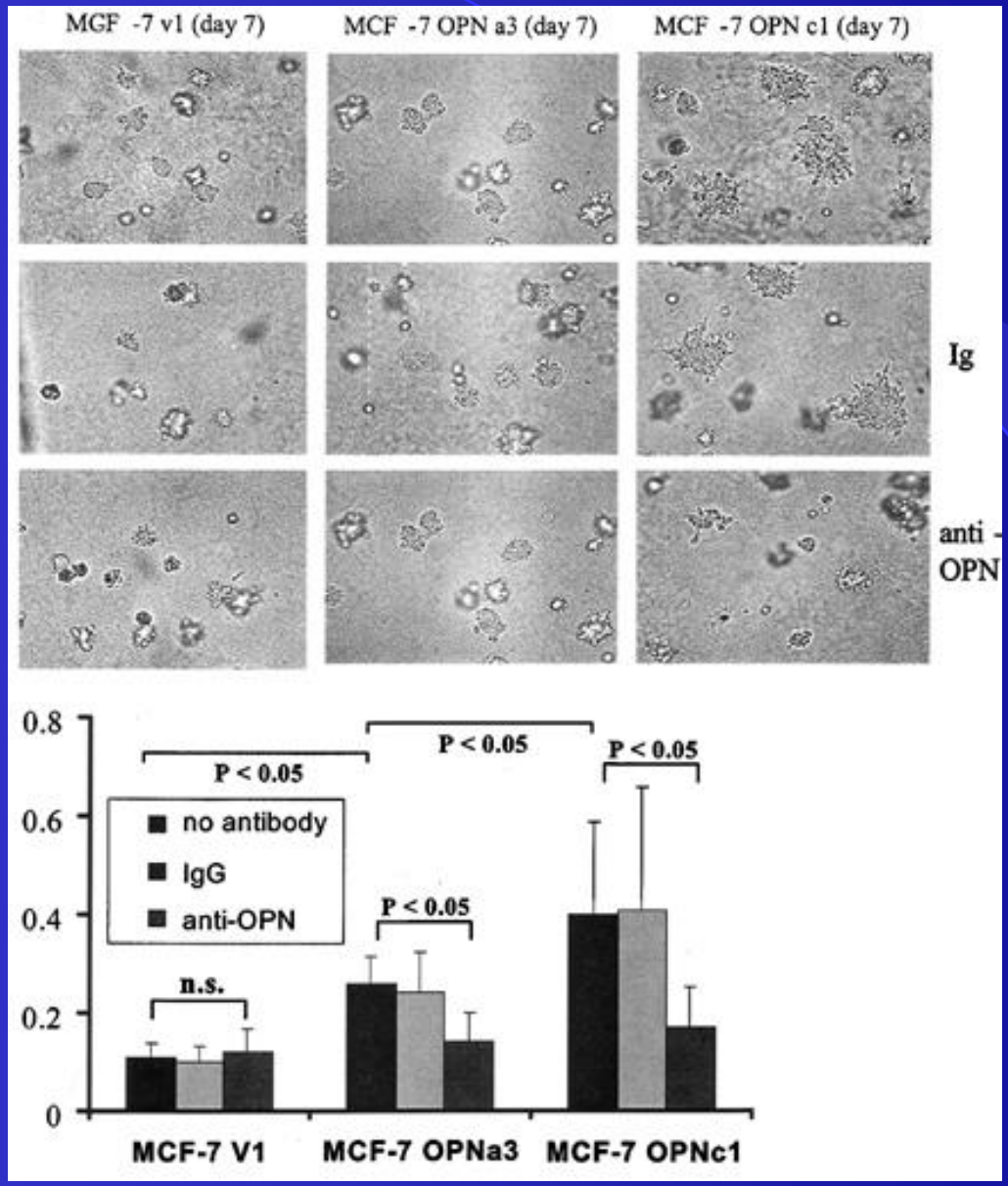
- Differentiated (untransformed) non-hematopoietic cells die by anoikis after losing contact with their substratum.
- Anchorage independent survival is essential for metastasis formation.
- Epithelial cell detachment → loss of glucose transport → ATP deficiency → anoikis, needs to be overcome in cancer progression.
- Elevated mitochondrial activity, oxygen consumption, ATP production → increased cancer invasiveness.
- Osteopontin is a cytokine and metastasis protein. The splice variant -c supports anchorage independence.


OSTEOPONTIN-C
SUPPORTS
ANCHORAGE-
INDEPENDENCE

STRUCTURE OF OSTEOPONTIN SPLICE VARIANTS



SPLICED OSTEOPONTIN SUPPORTS ANCHORAGE INDEPENDENCE

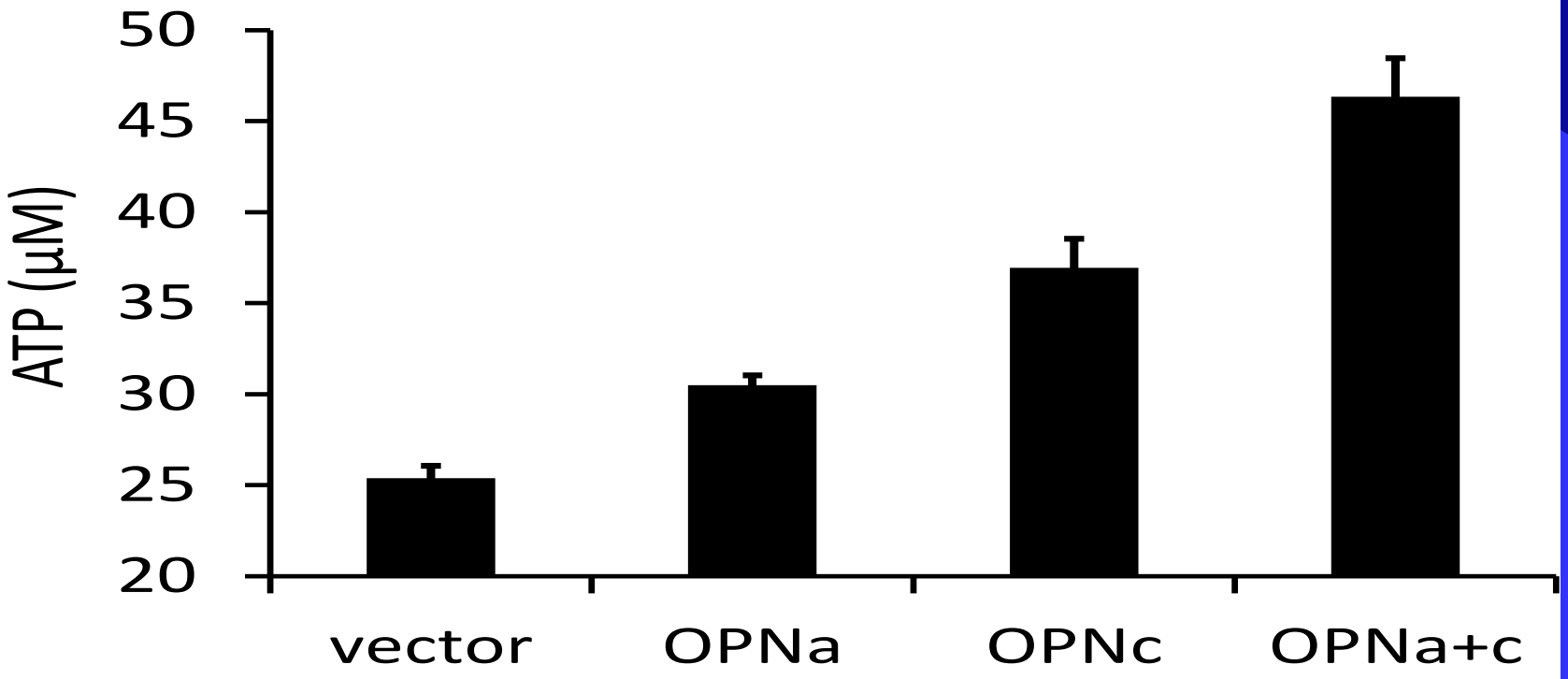


He et al. Oncogene 2006;25:2192-2202

ATP GENERATION IN DEADHERENT CELLS

p values

	vector	*OPNa*	*OPNc*
OPNa	0.019		
OPNc	0.0001	0.015	
OPNa+c	<0.0001	<0.0001	0.0008



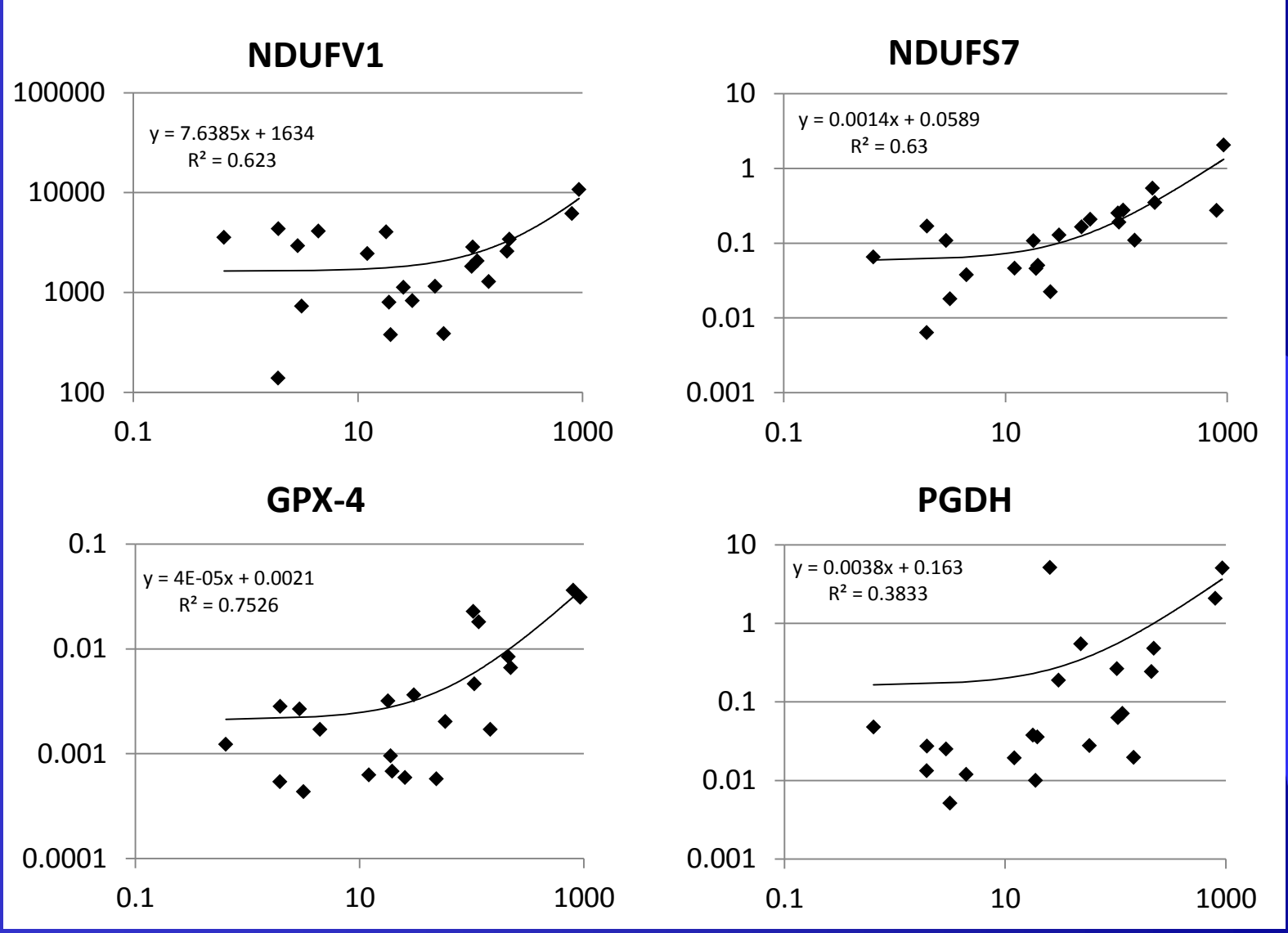
ANCHORAGE-INDEPENDENCE IS MEDIATED BY OXIDOREDUCTASES

Gene Category	List Hits	Popula-tion Hits	Probability	Benjamini FDR	Within System FDR	Genbank
oxidoreductase	22	507	2.49×10^{-3}	2.85×10^{-1}	7.14×10^{-2}	AF080577; *AF258341*; AK055328; BC015041; NM_000159; *NM_000284*; NM_000786; NM_000903; *NM_002085*; *NM_002134*; NM_002495; NM_003142; NM_003366; NM_004718; NM_005005; *NM_007103*; *NM_014222*; *NM_015974*; NM_016576; *NM_016593*; *NM_017732*; NM_025159
electron transporter	15	295	2.83×10^{-3}	2.85×10^{-1}	0	*AF258341*; BC000975; BC009037; NM_000903; *NM_002085*; *NM_002134*; NM_002495; NM_004718; NM_005005; *NM_006150*; *NM_007103*; NM_014222; *NM_016593*; NM_024701; X74794
monovalent inorganic cation transporter	10	120	3.76×10^{-4}	1.10×10^{-1}	0	BC008861; *NM_001687*; NM_002495; NM_003366; *NM_003945*; *NM_004047*; NM_004718; NM_005005; *NM_007103*; NM_014222

He et al. Oncogene 2006; 25:2192-2202

Weber et al. In: Ruffolo, Feuerstein, Hunter, Poste, Metcalf (Eds). Inflammatory Cells and Mediators in CNS Diseases. Amsterdam 1999;97-112

OSTEOPONTIN EXXPRESSION CORRELATES WITH OXIDOREDUCTASES

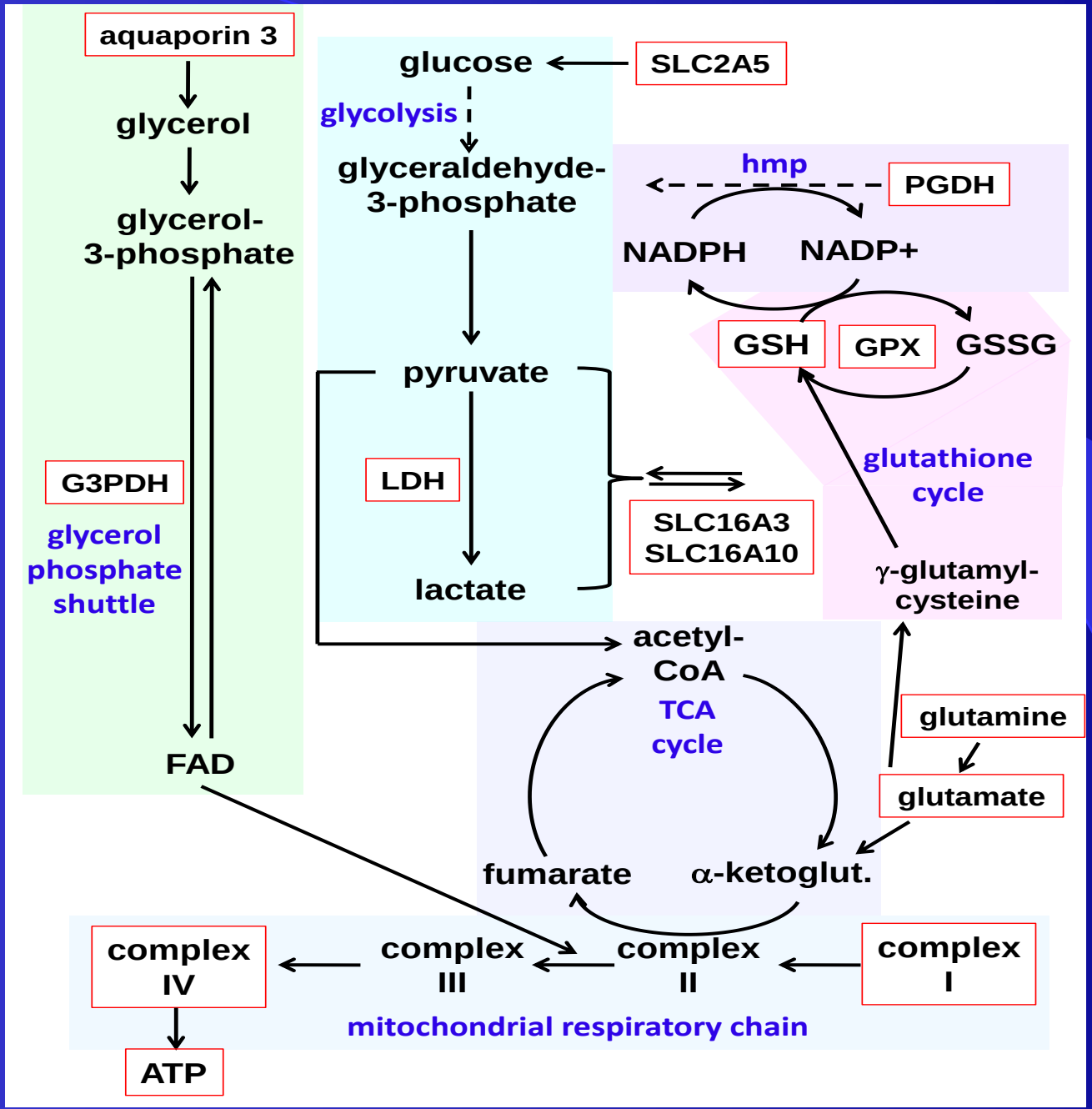


METABOLITES INDUCED BY OSTEOPONTIN-C

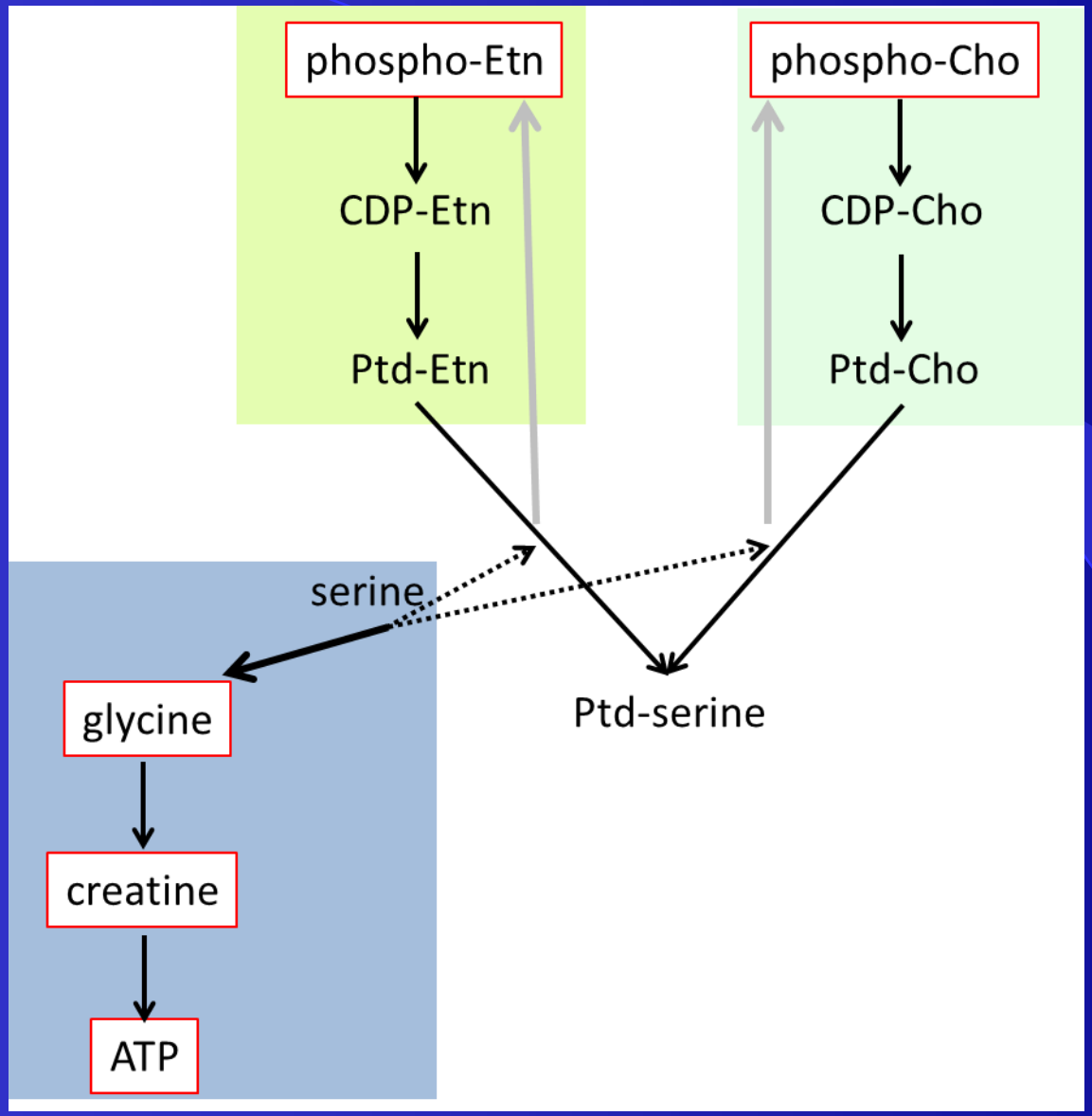
Metabolites	ppm	C concentration estimate (mM)	C STD	V concentration estimate (mM)	V STD	fold-change	P value
Creatine	3.04	0.161	0.003	0.130	0.003	1.24	*3.89E-09*
	3.93	0.223	0.014	0.182	0.017	1.23	9.76E-04
Glycine	3.56	0.283	0.006	0.253	0.007	1.12	*9.61E-06*
Glutamine	2.15	0.157	0.006	0.138	0.005	1.14	*8.11E-05*
	2.16	0.160	0.007	0.143	0.008	1.12	3.43E-03
Glutamate	2.35	0.125	0.006	0.110	0.006	1.13	6.49E-03
	2.36	0.134	0.005	0.117	0.007	1.14	3.95E-03
O-Phosphoethanolamine	3.98	0.073	0.009	0.048	0.011	1.54	1.41E-03
	4.00	0.090	0.012	0.051	0.009	1.78	3.95E-03
O-Phosphocholine	3.23	0.522	0.158	0.355	0.025	1.47	3.95E-03
Taurine	3.28	0.356	0.011	0.376	0.004	-1.06	1.61E-03
	3.42	0.207	0.003	0.233	0.003	-1.12	3.95E-03
	3.43	0.178	0.011	0.205	0.011	-1.15	1.99E-03

Shi et al. PlosOne 2014;9:e105675

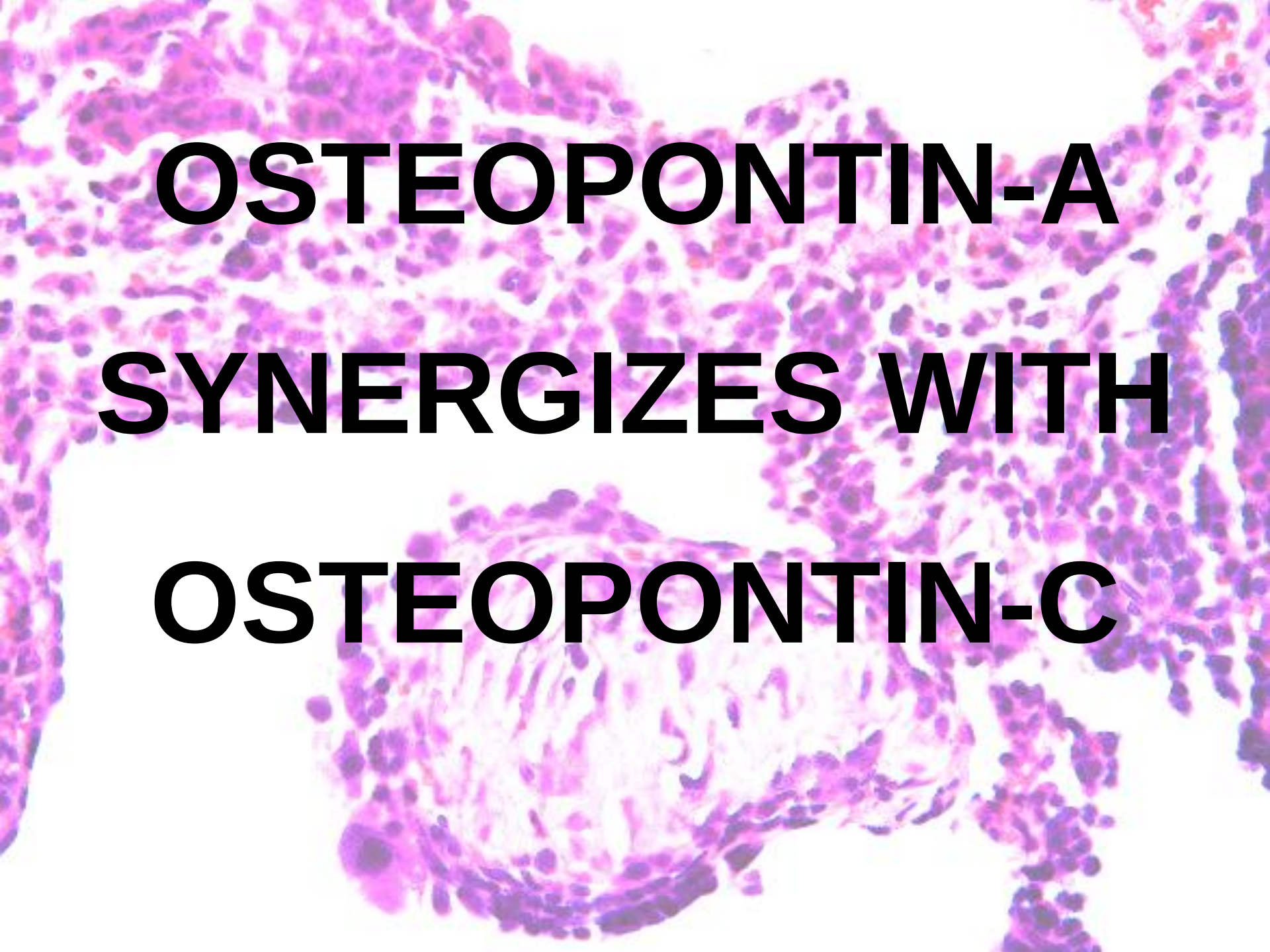
PATHWAYS AFFECTED BY OSTEOPONTIN-C



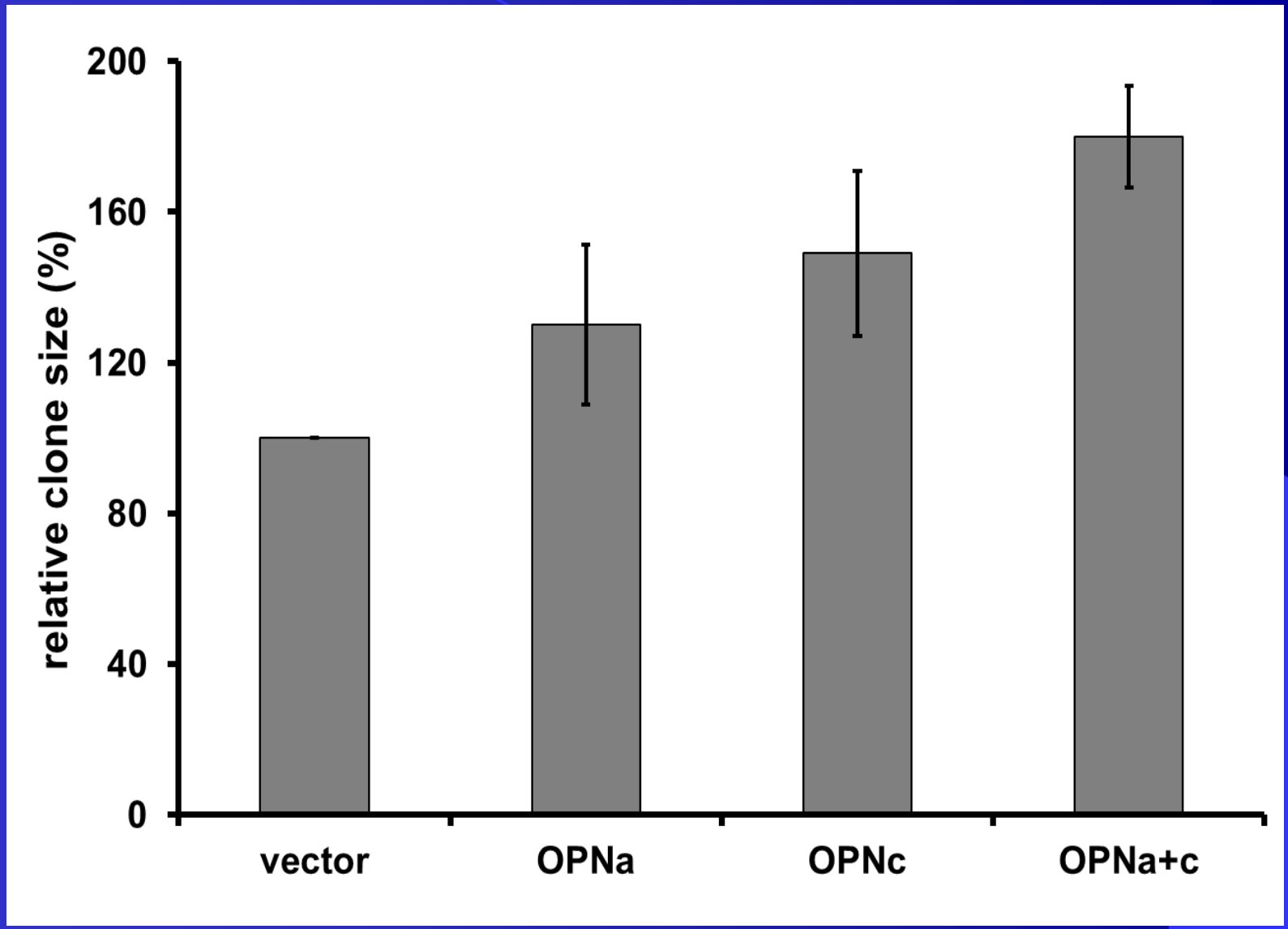
PATHWAYS AFFECTED BY OSTEOPONTIN-C




Osteopontin-c supports anchorage-independence by activating the energy metabolism


OSTEOPONTIN-A
SYNERGIZES WITH
OSTEOPONTIN-C

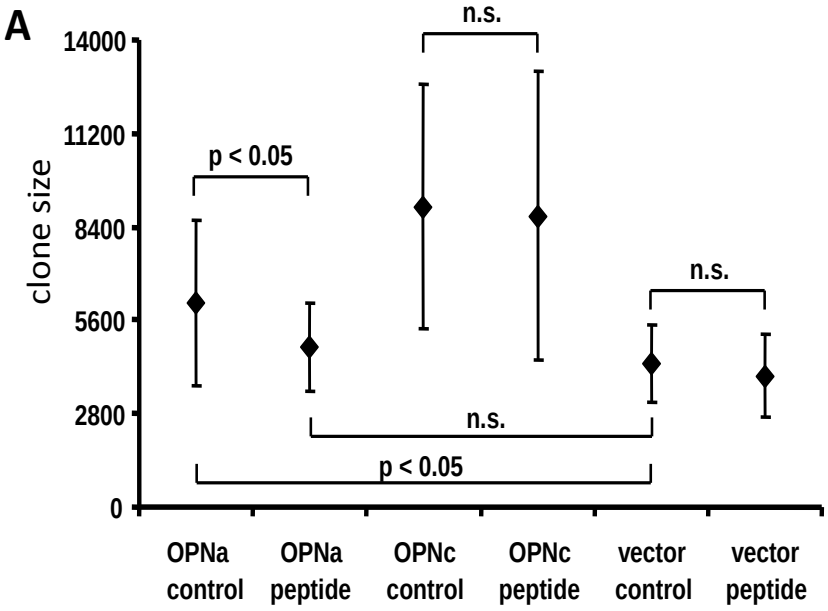
OSTEOPONTIN-A AND -C SYNERGIZE IN ANCHORAGE-INDEPENDENCE

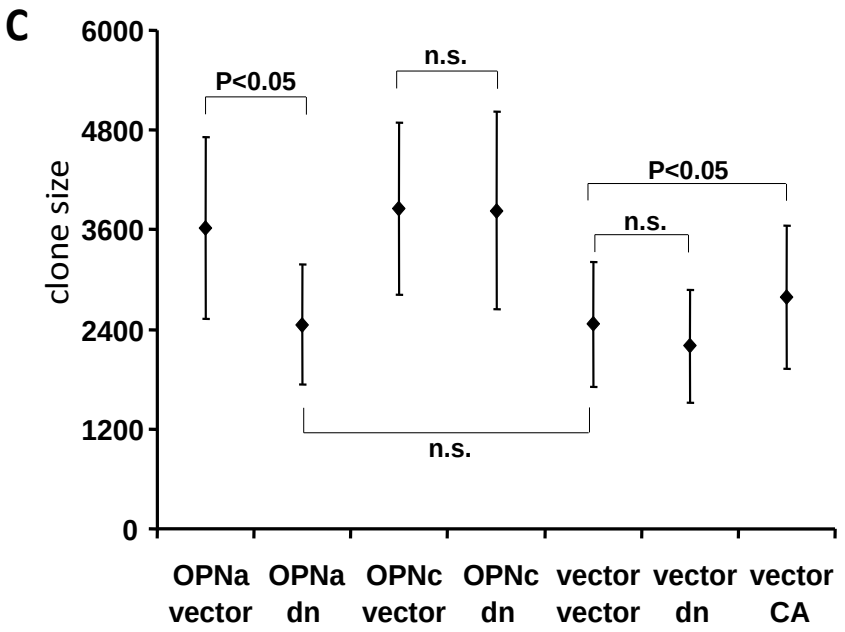


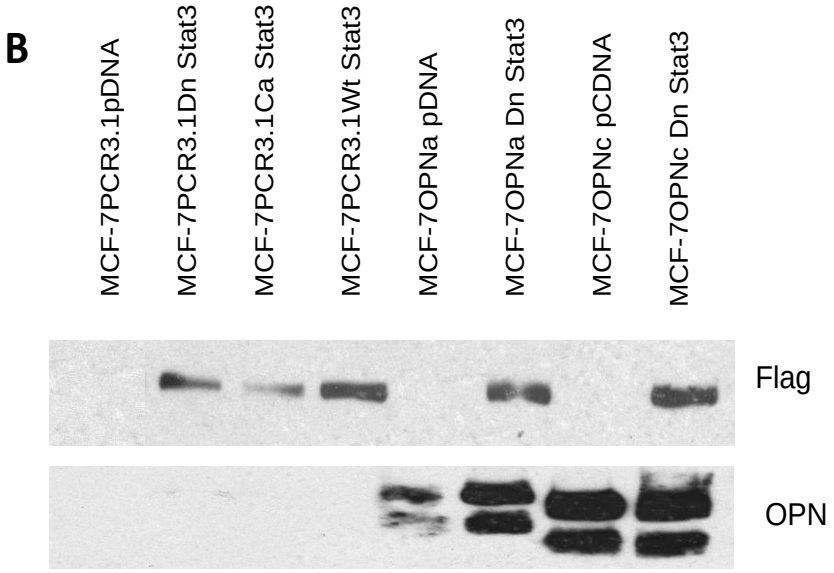
Shi et al. Cancer Letters,2014; 344:47-53

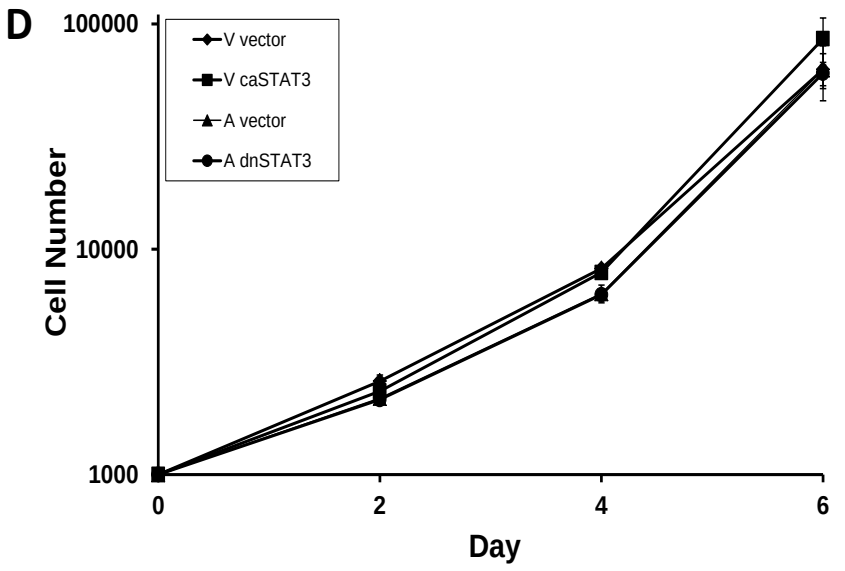
METABONOMICS OF ANCHORAGE-INDEPENDENCE

Metabolites	ppm	A concentration estimate (mM)	A STD	V concentration estimate (mM)	V STD	fold-change	P value
Creatine	3.04	0.114	0.001	0.130	0.003	-1.14	*1.09E-07*
Glucose	3.41	0.088	0.007	0.070	0.007	1.26	1.05E-03
	3.48	0.056	0.007	0.043	0.007	1.31	7.27E-03
	3.53	0.039	0.001	0.032	0.002	1.21	*4.93E-05*
	3.55	0.054	0.002	0.045	0.003	1.20	*1.07E-04*
	3.7	0.103	0.003	0.094	0.004	1.10	1.92E-03
	3.72	0.059	0.004	0.047	0.003	1.25	*1.24E-04*
	3.73	0.073	0.004	0.058	0.004	1.27	3.95E-03
	3.74	0.078	0.006	0.062	0.006	1.26	6.95E-04
	3.83	0.080	0.003	0.064	0.004	1.25	*2.39E-05*
	3.85	0.072	0.003	0.058	0.003	1.23	*1.17E-05*
	3.86	0.062	0.002	0.053	0.003	1.18	*1.49E-04*
Glutathione	2.17	0.133	0.004	0.156	0.005	-1.18	*2.71E-06*
	2.18	0.086	0.002	0.107	0.004	-1.25	*1.68E-07*
	2.54	0.045	0.004	0.056	0.004	-1.23	6.83E-04
Lactate	1.33	0.512	0.018	0.588	0.028	-1.15	*2.37E-04*
	4.1	0.062	0.002	0.069	0.002	-1.12	*5.40E-05*
	4.11	0.179	0.003	0.200	0.007	-1.12	*6.28E-05*
sn-Glycero-3-phosphocholine	3.24	0.846	0.018	0.774	0.027	1.09	3.09E-04
	3.68	0.165	0.007	0.152	0.006	1.08	6.00E-03
	3.69	0.115	0.004	0.104	0.005	1.11	1.02E-03
Taurine	3.42	0.205	0.010	0.233	0.003	-1.14	3.95E-03
	3.43	0.165	0.006	0.205	0.011	-1.25	*1.32E-05*


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vector control
vector peptide

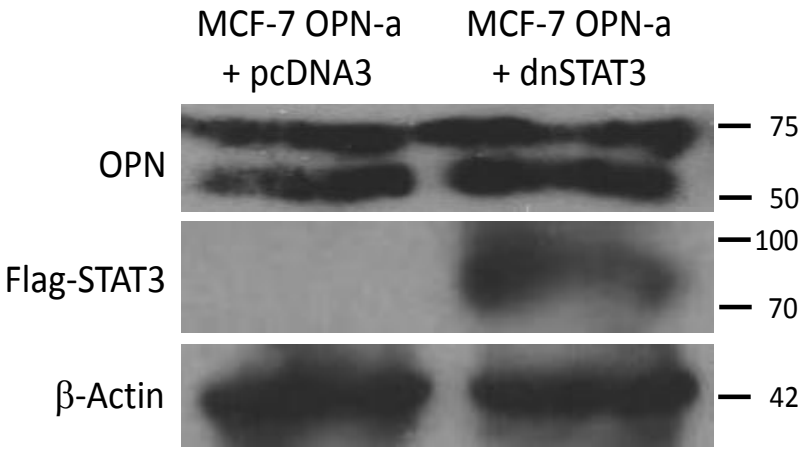

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OPNc vector
OPNc dn
vector vector
vector dn
vector CA


B
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MCF-7PCR3.1Wt Stat3
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MCF-7OPNc pCDNA
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Flag
OPN


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V caSTAT3
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STAT3 IS FUNCTIONALLY IMPORANT IN METABOLIC CHANGES

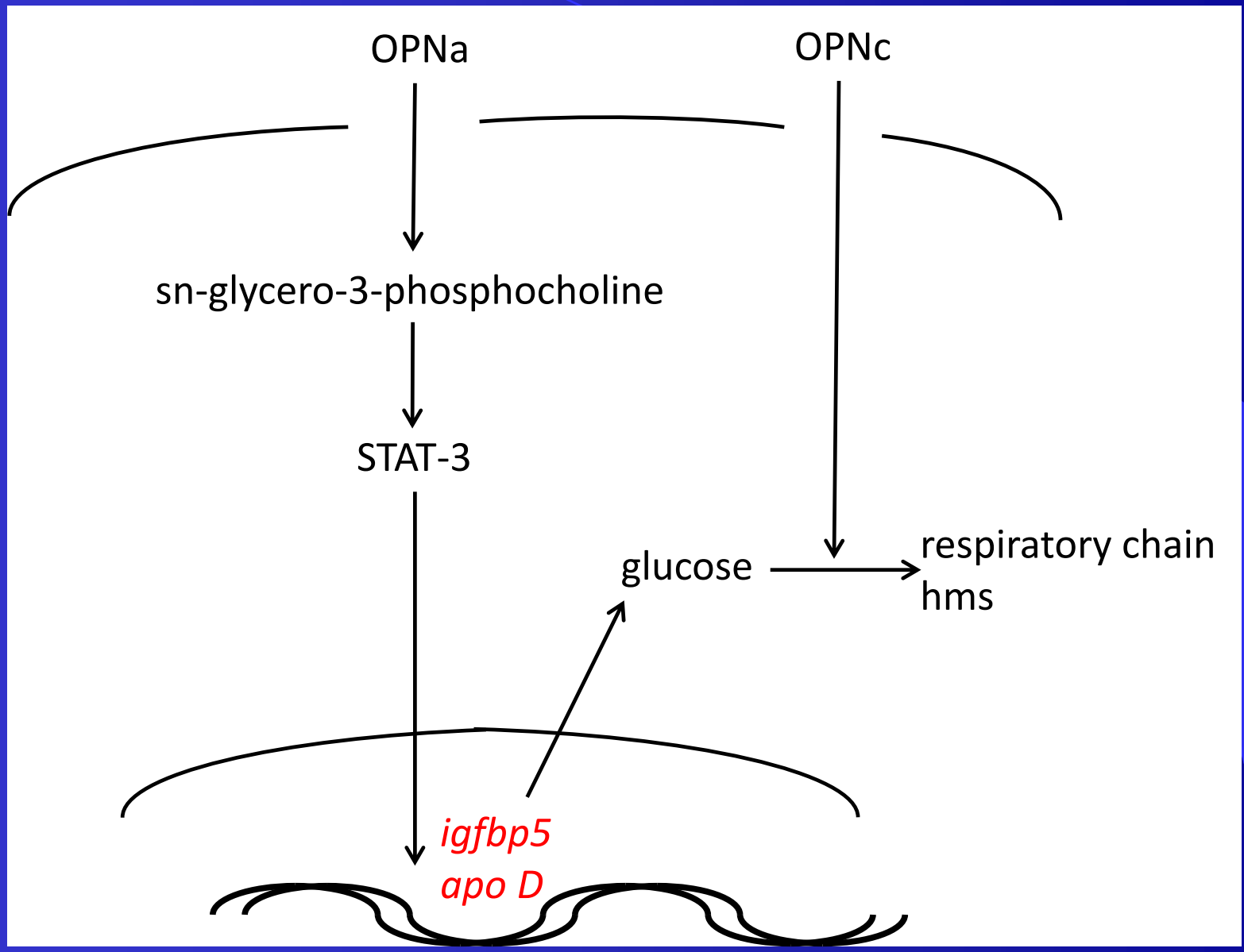
A



B

Metabolites	ppm	Fold change	P value
Lactate	1.34	1.23	*3.22E-04*
	4.13	1.17	*3.42E-04*
	4.11	1.17	9.96E-04
	4.12	1.14	1.25E-03
	4.10	1.20	3.95E-03
sn-glycero-3-phosphocholine	3.69	-1.15	*4.57E-04*
	3.684	-1.11	1.90E-03
	3.63	-1.12	2.26E-03
	3.68	-1.15	3.46E-03
	3.23	-1.11	2.32E-02
AMP	8.61	1.41	9.31E-04
Glucose	4.66	-1.41	5.40E-02
	4.65	-1.37	1.32E-01

OSTEOPONTIN SPLICE VARIANTS SYNERGIZE TO MEDIATE ANCHORAGE-INDEPENDENCE



OPN-a/OPN-c SYNERGISM IN METASTASIS

- osteopontin-a → STAT3, IGFBP5, apolipoprotein D → glucose homeostasis
- osteopontin-a dependent glucose regulation is a novel pathway
- metabolic skewing by distinct osteopontin splice variants: a new facet to metastasis gene function
- anchorage independence may require metabolic adjustment
- the autocrine secretion of osteopontin variants mediates this essential pro-metastatic effect

In anchorage-independent cancer cells, osteopontin-a stimulates the generation of glucose, which is used by Osteopontin-c for energy generation

ANCHORAGE INDEPENDENT SURVIVAL AND EXPANSION ARE ACHIEVED THROUGH METABOLIC ADJUSTMENT WITHIN THE DEADHERENT CELLS

PRO-METASTATIC LIGANDS ADJUST, OR SKEW, THE METABOLISM

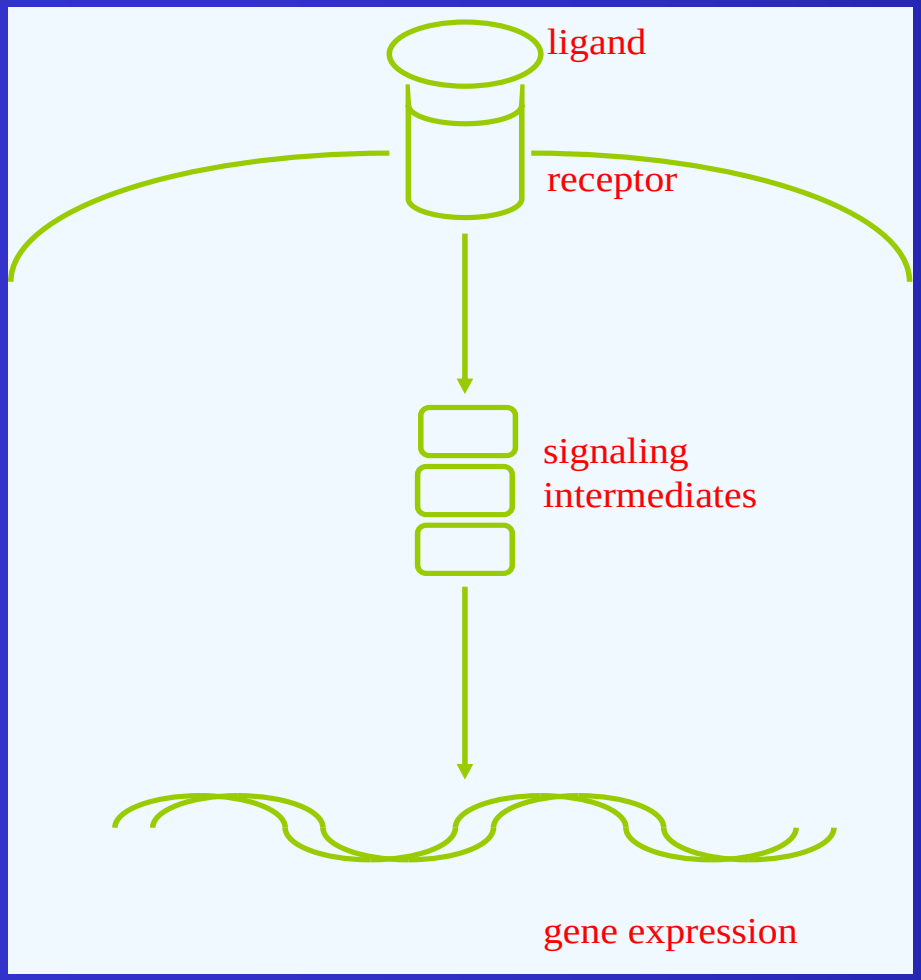
DISTINCT METABOLIC CHANGES ARE INDUCED BY OSTEOPONTIN-A AND -C

GLUCOSE UPTAKE (OSTEOPONTIN-A) AND UTILIZATION (OSTEOPONTIN-C) SATISFY THE ENERGY REQUIREMENT BY CANCER CELLS IN CIRCULATION

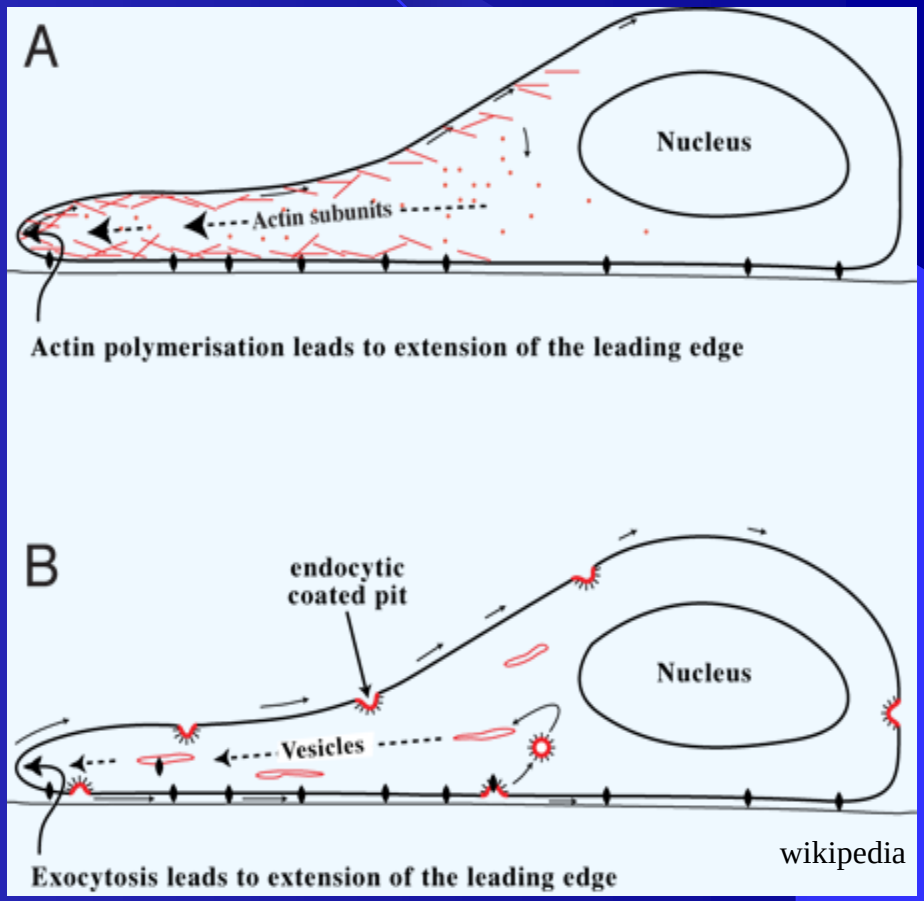
METABOLIC RESPONSES TO ENVIRONMENTAL CUES ARE LIKELY MORE COMMON IN CELL BIOLOGY THAN HAS HITHERTO BEEN RECOGNIZED

SIGNAL TRANSDUCTION

conventional: cells communicate environmental cues via receptors to their nuclei to induce adaptive changes in gene expression.



cancer metastasis: epigenetic regulation of directed migration (homing), penetration of tissue barriers (invasion), survival in the circulation (anti-anoikis).



- Signaling research has elucidated how cells communicate environmental cues via receptors to their nuclei and induce gene expression changes. Cell responses based on epigenetic mechanisms are less well understood
- Signaling in cancer metastasis is associated with directed cell migration (homing), penetration of tissue barriers (invasion), and survival of cells in the circulation (anti-anoikis). These functions have a strong epi-genetic component
- The response of deadherent cells to pro-metastatic ligands (such as osteopontin splice variants) involves metabolic adjustment
- The increased glucose uptake by osteopontin-a may support increased energy requirements by deadherent cancer cells

- Osteopontin- c promotes tumor progression. It supports anchorage-independence and increases energy generation, manifested in elevated ATP levels
- an upregulation of the energy metabolism after deadhesion is important in tumor progression, but is absent from untransformed cells (that do not produce osteopontin-c)
- Osteopontin-c is never expressed without the full-length form osteopontin-a. Each splice variant activates signal transduction pathways that are distinct from the other
- There is a coalescence in cancer cells between osteopontin-a, which increases the cellular glucose levels, and osteopontin-c, which utilizes this glucose to generate energy
- The splice form-specific metabolic effects of osteopontin add a novel aspect to the pro-metastatic functions of this molecule