METABOLIC REPROGRAMING AND ANGIOGENESIS IN CANCER

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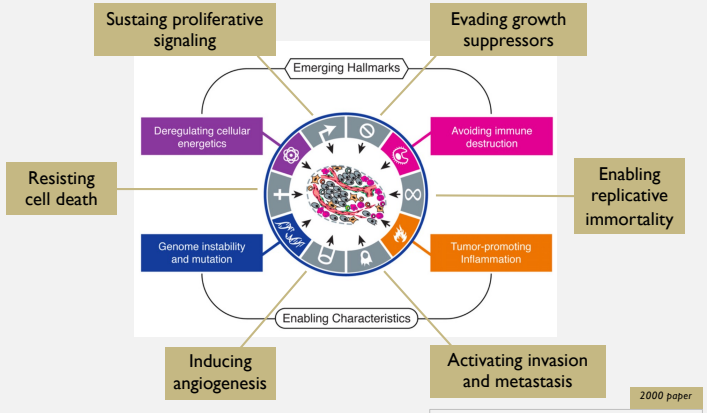
HALLMARKS OF CANCER



Hanahan & Weinberg, Cell, 2011, 144:646.

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METABOLIC STRESS: CAUSES AND CONSEQUENCES

Blood flux decrease
↘ Oxygen
↘ Nutrients

Nutritional & metabolic needs increase

In the core of the tumor

To support uncontrolled cell growth

Metabolic stress + Cell's adaptive responses

Neo-angiogenesis

Poorly effective neo-angiogenesis due to abnormal blood vessels and frequent collapsus → sustained pro-angiogenic signaling

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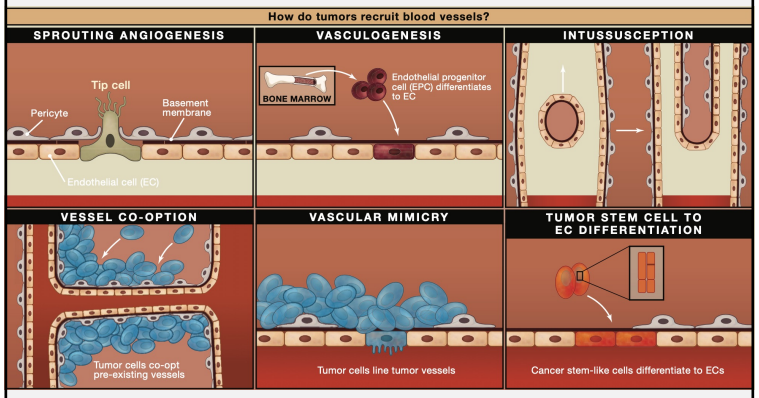
ANGIOGENESIS



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Angiogenesis drivers

Balance of pro- and anti-angiogenic factors

Pro-angiogenic factors	Anti-angiogenic factors
Acidic fibroblast growth factor (aFGF, FGF1)[b]	(A) Derived from the extracellular matrix
Angiogenin[b]	Anastellin
Angiopoietin-1	Arresten
Angiopoietin-2	Canstatin
Basic fibroblast growth factor (bFGF, FGF2)[b]	Chondromodulin-1
Ephrin-A1	EFC-XV
Ephrin-B1	Endorepellin
Ephrin-B2	
Epidermal growth factor (EGF)[b]	Endostatin
Granulocyte colony-stimulating factor (GCSF)	Fibulin fragments
Macrophage-granulocyte colony-stimulating factor (GM-CSF)	Thrombospondin-1 and -2
Hepatic growth factor (HGF, scatter factor)[b]	Tumstatin
Interleukin-8 (Il-8, CXCL8)[b]	(B) Non-matrix derived factors
Leptin	Angiostatin
Placental growth factor (PlGF)[b]	Antithrombin III (cleaved)
Platelet-derived endothelial growth factor (PD-EGF)[b]	Hemopexin-like domain (PEX)
Platelet-derived growth factor-A (PDGF-A)[b]	Interferon-α, -β, -γ
Platelet-derived growth factor-B (PDGF-B)[b]	Interleukin-1, -4, -12, -18
Transforming growth factor-α (TGF-α)[b]	2-Methoxyestradiol
Transforming growth factor-β (TGF-β)[b]	Pigment epithelium-derived factor (PEDF)
Tumor necrosis factor (TNF-α)[b]	Plasminogen kringle-5
Vascular endothelial growth factor (VEGF-A)[b]	Platelet factor-4
VEGF-B[b]	Prolactin fragments
VEGF-C[b]	Prothrombin kringle-2
VEGF-D[b]	Semaphorin-3F
	Soluble VEGFR1
	TIMP-2
	Troponin I
	TrpRS
	Vasostatin

Roskoski, Crit Rev Oncol Hematol. 2007, 62:179.

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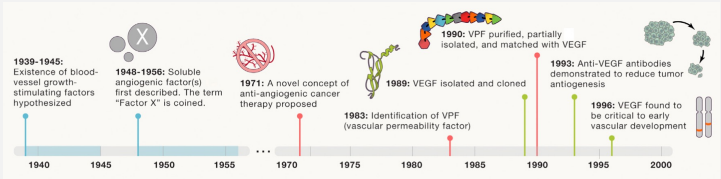
Angiogenesis drivers

Vascular Endothelial Growth Factor

Timeline of VEGF discovery



Apte et al., Cell. 2019, 176:1248.

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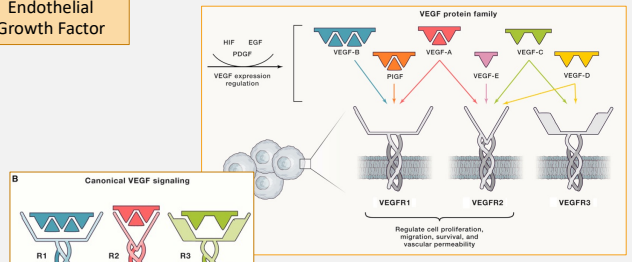
Angiogenesis drivers

Vascular Endothelial Growth Factor

A complex signaling system of 3 Receptors and 6 ligands...



Apte et al., Cell. 2019, 176:1248.

High expressions of:
→ VEGF-A by cancer cells
→ VEGFR-1 and VEGFR-2 in endothelial cells

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Angiogenesis drivers

Vascular Endothelial Growth Factor

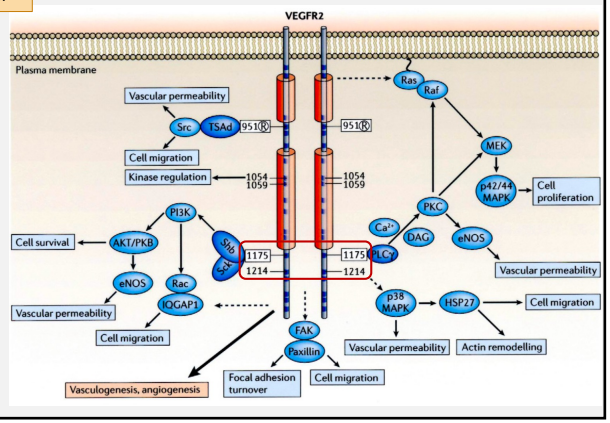
...with pleiotropic effects

Ligand binding → Receptor dimerization and trans-auto phosphorylation of Tyr residues

Major P-Tyr residues

Adaptor

2nd messenger



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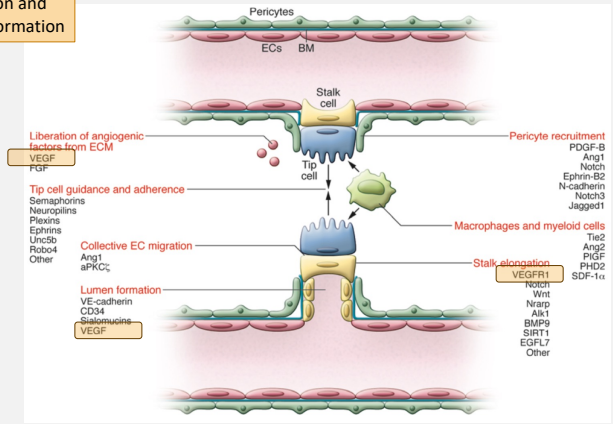
Angiogenesis

Coordinated proliferation, migration and junction formation



Welti et al., J. Clin. Invest. 2013, 123:3190-3200

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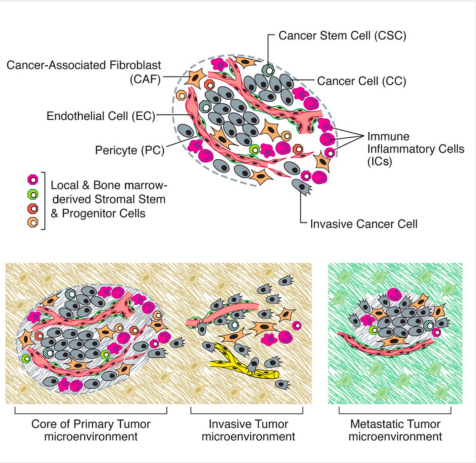
METABOLIC STRESS RESPONSE:
COMPLEX INTERPLAY BETWEEN TUMOUR CELLS

Heterogeneity of the tumor micro-environment



Hanahan & Weinberg, Cell, 2011, 144:646.

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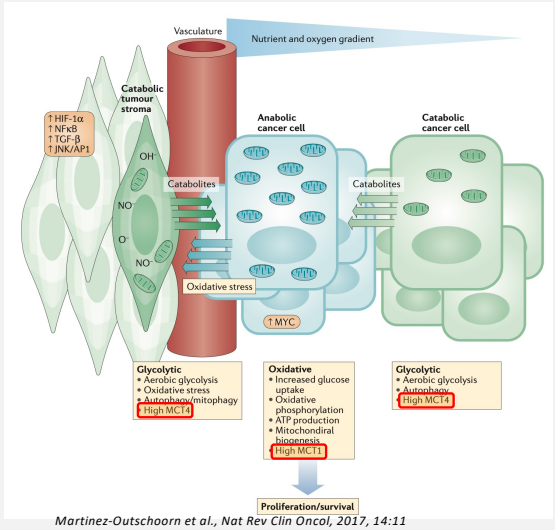
Heterogeneity of cell responses

Catabolic cancer and stromal cells

+

Anabolic cancer cells

MCT = Mono-carboxylate transporter



Martinez-Outschoorn et al., Nat Rev Clin Oncol, 2017, 14:11

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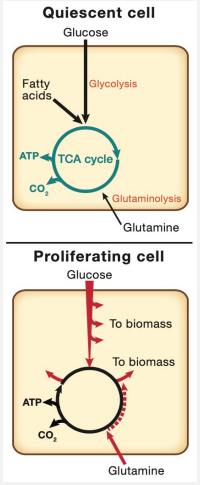

Proliferating cells rewire their metabolism to support growth

Quiescent cells efficiently convert nutrients into energy to sustain basic cellular processes:

- breakdown of fuels such as glucose, glutamine, and fatty acids for complete oxidation to carbon dioxide through the tricarboxylic acid cycle (TCA cycle).
- The chemical energy of these fuels is harnessed by reducing electron carriers NAD+ and FAD to NADH and FADH2, respectively.
- These high-energy electrons are used to generate large amounts of adenosine triphosphate (ATP) by the mitochondrial electron transport chain.

Rapidly proliferating cells must generate the proteins, lipids, and nucleic acids necessary to create a daughter cell.

- Take up of large amounts of nutrients such as glucose and glutamine that are used to support cell growth.
- Intermediates of glycolysis and the TCA cycle provide the building blocks for nucleic acids, amino acids, and fatty acids.
- Glutamine catabolism maintains a steady supply of nutrients into the TCA cycle (anaplerosis), thus preserving the TCA cycle integrity despite continual efflux of metabolites to support growth.

Finley et al., Cell Metab., 2013, 17:466.

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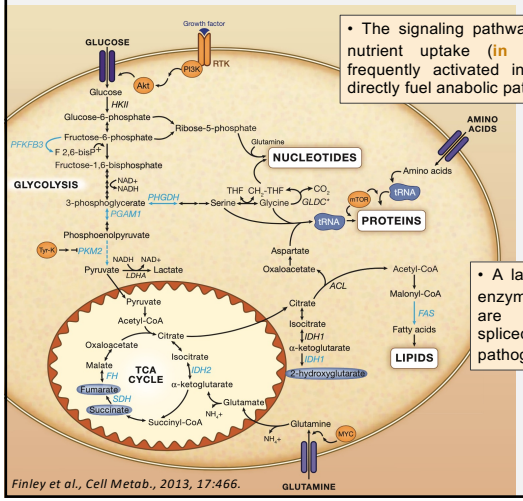
To grow and divide, cells must be able to synthesize nucleotides, proteins, and lipids from exogenous glucose, glutamine, and essential amino acids.



• The signaling pathways controlling nutrient uptake (**in orange**) are frequently activated in cancer and directly fuel anabolic pathways.

• A large number of metabolic enzymes (**highlighted in blue**) are amplified, alternatively spliced, or mutated in the pathogenesis of cancer

Finley et al., Cell Metab., 2013, 17:466.

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SIGNALING AND ADAPTIVE RESPONSES

The Warburg effect

Otto Warburg (1956):
Cancer cells unexpectedly produce lactic acid in glycolysis, even in the presence of oxygen

In normal cells, glycolysis produces pyruvic acid which enters mitochondria and undergoes complete oxidation into CO_2 in the presence of oxygen with consequent production of ATP via oxidative phosphorylation (OXPHOS).

→ ATP is a potent negative feedback regulator of glycolysis via inhibition of the rate-limiting enzyme phosphofructokinase- 1 in the glycolytic pathway.
→ Therefore lactic acid is produced in normal cells only in conditions of hypoxia.

Since their publication, Warburg's findings in cancer cells were generally thought to represent a symptom of cancer rather than the primary cause.

This notion has changed dramatically in the past two decades

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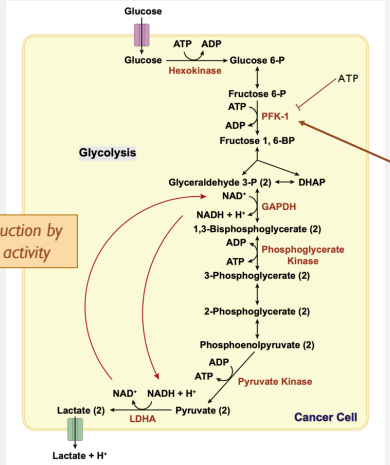
Metabolic mechanisms

Coupling of Glycolysis with Lactate production



Increased NAD+ production by LDH sustains GAPDH activity

Fructose-2,6 bisphosphate

- *Elevated in cancer cells*
- *Allows PFK-1 to function in the presence of ATP*

Brown & Ganapathy, Pharmacol. Therap, 2019, https://doi.org/10.1016/j.pharmthera.2019.107451

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Signaling mechanisms

HIF-1 signaling

Heterodimeric transcription factor

HIF-1 α + HIF-1 β

- Oxygen-sensitive factor
- Inducible

- = Aryl-Hydrocarbon Receptor (AhR) Nuclear Translocator
- Constitutive

→ Very short half-life (~5 min) in normoxia
→ Degraded by pVHL / Ubiquitin / Proteasome pathway

O_2 favors HIF-1α proline hydroxylation

Recognition by the von Hippel-Lindau protein
= Tumor suppressor that belongs to an E3 ubiquitin-protein ligase

HIF-1α poly-ubiquitination

Proteasomal degradation

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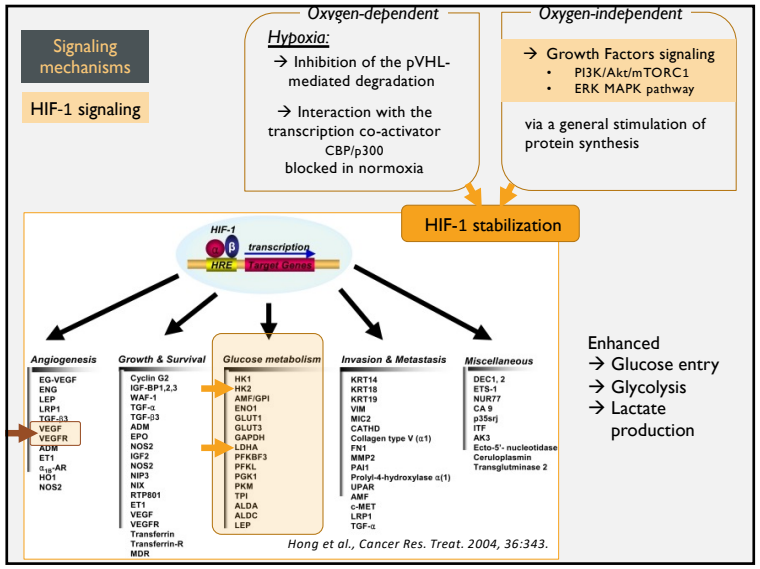
Signaling mechanisms
HIF-1 signaling
Oxygen-dependent
Hypoxia:
→ Inhibition of the pVHL-mediated degradation
→ Interaction with the transcription co-activator CBP/p300 blocked in normoxia
Oxygen-independent
→ Growth Factors signaling
• PI3K/Akt/mTORC1
• ERK MAPK pathway
via a general stimulation of protein synthesis
HIF-1 stabilization
HIF-1
transcription
HRE
Target Genes
Angiogenesis
Growth & Survival
Glucose metabolism
Invasion & Metastasis
Miscellaneous
Enhanced
→ Glucose entry
→ Glycolysis
→ Lactate production
Hong et al., Cancer Res. Treat. 2004, 36:343.

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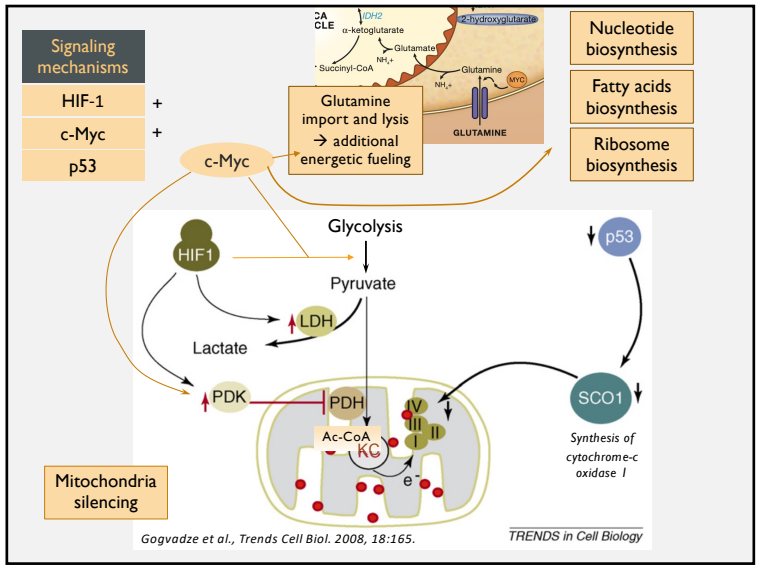
Signaling mechanisms
HIF-1 +
c-Myc +
p53
Nucleotide biosynthesis
Fatty acids biosynthesis
Ribosome biosynthesis
Glutamine import and lysis → additional energetic fueling
GLUTAMINE
c-Myc
Glycolysis
HIF1
Pyruvate
LDH
Lactate
PDK
PDH
p53
SCO1
Ac-CoA
KC
Synthesis of cytochrome-c oxidase I
Mitochondria silencing
Gogvadze et al., Trends Cell Biol. 2008, 18:165.
TRENDS in Cell Biology

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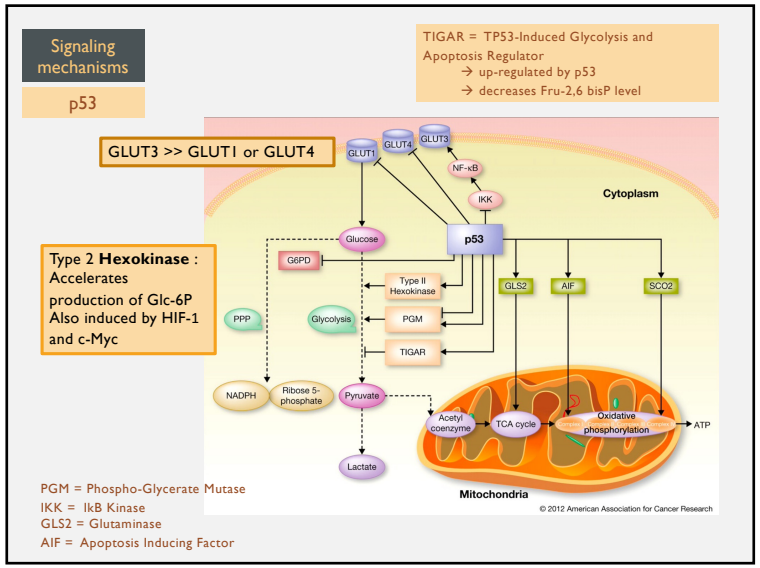
Signaling mechanisms
p53
TIGAR = TP53-Induced Glycolysis and Apoptosis Regulator
→ up-regulated by p53
→ decreases Fru-2,6 bisP level
GLUT3 >> GLUT1 or GLUT4
Type 2 Hexokinase : Accelerates production of Glc-6P Also induced by HIF-1 and c-Myc
Cytoplasm
Mitochondria
PGM = Phospho-Glycerate Mutase
IKK = IkB Kinase
GLS2 = Glutaminase
AIF = Apoptosis Inducing Factor

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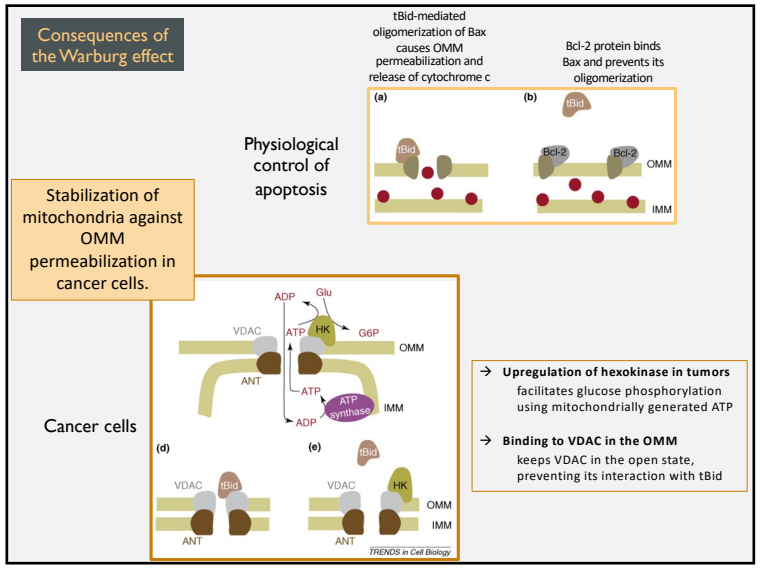
Consequences of the Warburg effect
tBid-mediated oligomerization of Bax causes OMM permeabilization and release of cytochrome c
Bcl-2 protein binds Bax and prevents its oligomerization
Physiological control of apoptosis
Stabilization of mitochondria against OMM permeabilization in cancer cells.
Cancer cells
→ Upregulation of hexokinase in tumors facilitates glucose phosphorylation using mitochondrially generated ATP
→ Binding to VDAC in the OMM keeps VDAC in the open state, preventing its interaction with tBid
TRENDS in Cell Biology

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Consequences of the Warburg effect

Release of Reactive Oxygen Species (ROS)

Mitochondrial respiration by-product

Also increased by hypoxia

Approx. 2% of the O_2 consumed by respiration yields superoxide anion

ROS (balanced by anti-oxidants)

In case of imbalance: oxidative stress due to macromolecule damage

↓

mt DNA mutations

↓

Mitochondria malfunction & contribution to OXPHOS silencing

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Consequences of the Warburg effect

Lactate production

Lactate DeHydrogenase (LDH)

Two subunits : LDH-A & LDH-B assembled in a tetramer

HIF-1 + c-Myc

Expression of LDH-A

Normal cell → Cancer cell

LDH1 (B4)	LDH2 (B3A1)	LDH2 (A2B2)	LDH2 (A3B)	LDH5 (A4)

Affinity for lactate

Affinity for pyruvate

N.B. Isoenzyme profile modification in cancer cells also affects Pyruvate Kinase to favor a low-affinity PK M2 isotype → PEP accumulation and decrease of the glycolysis rate → favors Glc-6P / Fru-6P entry into the pentose phosphate pathway, the biosynthesis of NADPH,H+ and subsequently, that of nucleic acids.

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Consequences of the Warburg effect

Lactate export

Monocarboxylate Transporters (MCT)

→ MCT 1 & 4 highly upregulated in cancer

→ Function as H^+-driven transporters (symporters)

H^+ binds first → conformational change that allows lactate binding and release in the extracellular medium, then H^+ release

Normal cells : MCT-1 ***functions according to the transmembrane gradient of lactate***

Cancer cells : High Lactate →MCT-1 saturates → low-affinity MCT-4 exports lactate and H^+

↓

Regulation of intracellular and extracellular pH

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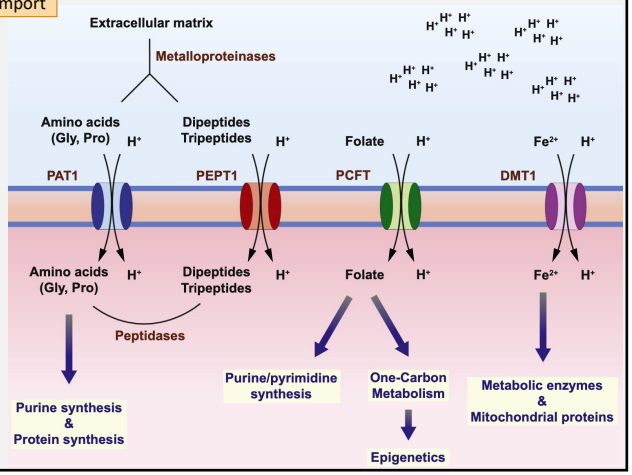
Consequences of the Warburg effect

Metabolite import

Proton gradient coupling of nutrient intake by cancer cells



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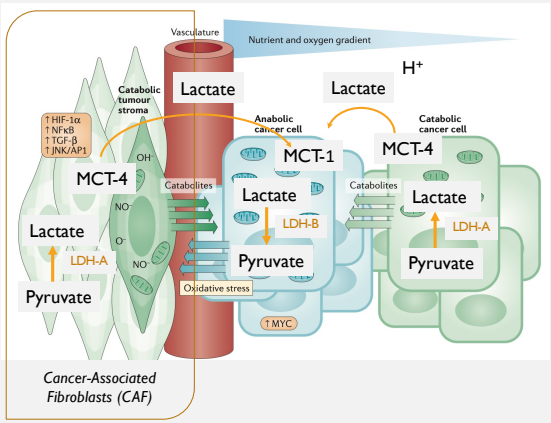
Consequences of the Warburg effect

Lactate shuttling in the tumor micro-environment



« Reverse » Warburg effect = *The fact that cancer cells instruct fibroblasts to become «catabolic »*

The mechanism is still elusive, however the early loss of caveolin-1 in CAF could be a primary step in allowing HIF-1-dependent control over MCT-4 and LDH-A expressions

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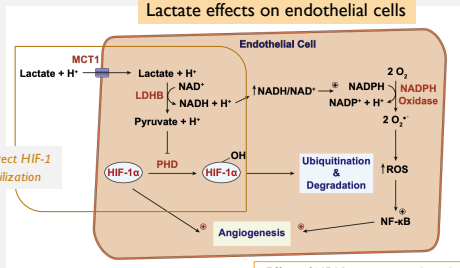
Consequences of the Warburg effect

Lactate shuttling in the tumor micro-environment

Lactate effects on endothelial cells



Indirect HIF-1 stabilization

Lactate use by macrophages

Decreased NAD+ levels

Poly-ADP-ribose polymerase (PARP) inhibition (NAD+-dependent)

Local VEGF production by macrophages

Effect of NF-kB via increased production of the pro-angiogenic cytokine IL-8

PHD = prolyl-hydroxylase

Other tumor-promoting effects of lactate

→ Dampening of the immune response
→ Activation of c-Myc via HIF-2α stabilization
→ Overexpression of TGF-β2 in cancer cells → MMP & ECM remodeling
→ HDACs inhibition, epigenetic modifications, stemness ....?

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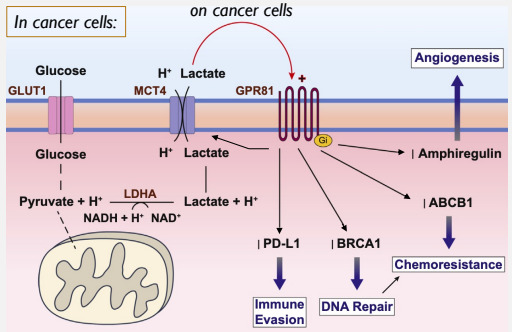
Consequences of the Warburg effect

Lactate as an extracellular messenger

Lactate signals via a specific GPCR

Autocrine and paracrine effects on cancer cells

In cancer cells:



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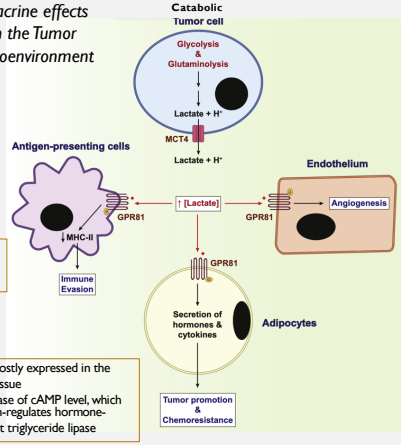
Consequences of the Warburg effect

Lactate as an extracellular messenger

Paracrine effects on the Tumor Microenvironment



GPR81 signaling limits Inflammation and causes innate immune cells inhibition.

GPR81 mostly expressed in the adipose tissue
→ Decrease of cAMP level, which also down-regulates hormone-dependent triglyceride lipase

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THERAPEUTIC TARGETING OF NEOANGIOGENESIS AND OF METABOLIC ADAPTATIONS

→ Powerful paradigm for tumor growth control
- Anti-angiogenic therapy

→ New field of anticancer therapy
- Control of metabolic adaptations and their consequences

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