

Oxygen regulated machinery plays an important role in cancer

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2019 Nobel Prize in Physiology or Medicine



William G. Kaelin, Jr.



Sir Peter J. Ratcliffe



Gregg L. Semenza

How cells sense and adapt to oxygen availability

2019 Nobel Prize in Physiology or Medicine



William G. Kaelin, Jr.

- Born – 1957 – New York
- M.D. – Duke University, Durham
- Specialist Training in Internal Medicine and **Oncology**
 - John Hopkins University, Baltimore
 - Dana-Farber Cancer Institute, Boston
- Own research lab – Dana-Farber Cancer Institute, Boston – 2002
- Professor – Harvard Medical School – 2002
- Investigator – Howard Hughes Medical Institute since 1998

2019 Nobel Prize in Physiology or Medicine



Sir Peter J. Ratcliffe

- Born – 1954 – Lancashire, UK
- Medicine – Gonville and Caius College at Cambridge University
- Specialist Training in Internal Nephrology – Oxford University
- Own research lab – Oxford University – 1996
- Director – Clinical Research, Francis Crick Institute, London
– Target Discovery Institute, Oxford University
- Member – Ludwig Institute for **Cancer** Research

2019 Nobel Prize in Physiology or Medicine



Gregg L. Semenza

- Born – 1956 – New York
- B.A. (Biology) – Harvard University, Boston
- M.D./Ph.D. – University of Pennsylvania, Philadelphia – 1984
- Specialist Training – Pediatrics – Duke University, Durham
- Post-doctoral training – John Hopkins University, Baltimore
- Own research lab – John Hopkins University, Baltimore
- Professor – John Hopkins University, Baltimore – 1999
- Director – Vascular Research Program
@John Hopkins Institute for Cell engineering since 2003

The term hypoxia has been extensively used to describe a state of insufficient oxygen, which can be present in tumours as well as normal tissues and wounds

Commonly used units for oxygen concentration

Units used to define pO_2	Conversion factors
Millimetre of mercury (mmHg)	$1 \text{ mmHg} = 0.13\% O_2, 133.3 \text{ Pa}$
Percentage oxygen (%)	$1\% = 7.6 \text{ mmHg}, 1.013 \text{ kPa}$

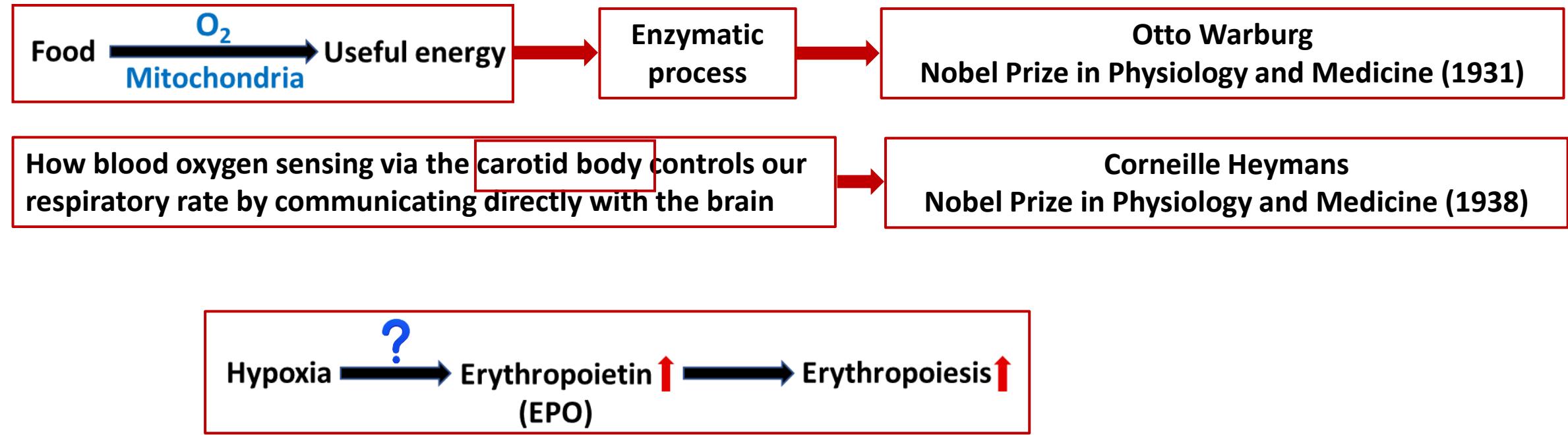
Measuring Tumour Hypoxia Using Positron Emission Tomography Imaging

Magnetic Resonance Imaging and Measurement of Hypoxia

Tissue	pO ₂ (mmHg)	% O ₂	Reference
Brain	35	4.6	[3]
Lung	42.8	5.6	[4]
Liver	31	4.08	[5]
Kidney	72	9.5	[6]
Muscle	25	3.25	[7]
Bone marrow	54.9	7.14	[8]
Skin	8–35	1.05–4.61	[9]
Intestine	61	8.03	[10]

Within any tumour, oxygen levels are extremely heterogeneous and can include mild hypoxia ($\leq 2\% \text{ O}_2$) and severe levels of hypoxia ($< 0.1\% \text{ O}_2$).

No O₂ – No animal life



How does O₂ control the process by itself?

Which cellular components mediate the process?

Semenza et al.

A protein complex that binds specific DNA – O₂ dependent

*hypoxia-inducible factor
(HIF)*

HIF-1 α

ARNT (HIF-1 β)

Low O₂ → High HIF-1 α → Binds EPO & other genes

Normal O₂ → Ubiquitination of HIF-1 α → Proteasomal degradation

Aaron Ciechanover, Avram Hershko and Irwin Rose - Nobel Prize in Chemistry (2004)

O₂ dependent binding of ubiquitin to HIF-1α?

William G. Kaelin, Jr.

von Hippel-Lindau's disease (VHL disease) – inherited VHL mutations – high risk of cancer

Non-functional VHL in cancer cells → hypoxia-regulated genes ↑

Re-introduction of VHL → Normal levels of hypoxia-regulated genes

Other groups

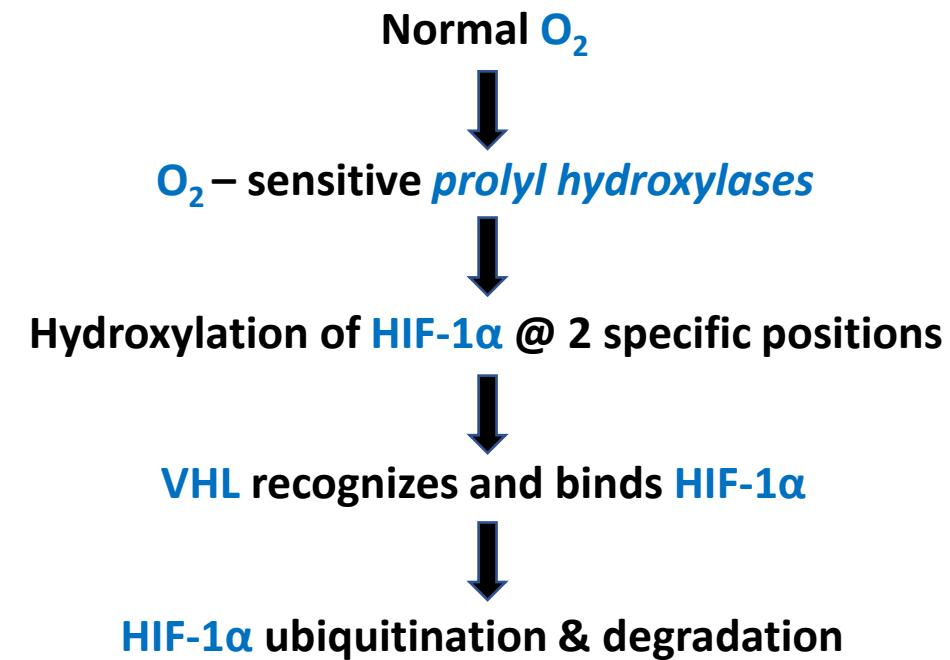
VHL (a component of ubiquitination complex) – labels proteins with ubiquitin, causes proteasomal degradation

Sir Peter J. Ratcliffe

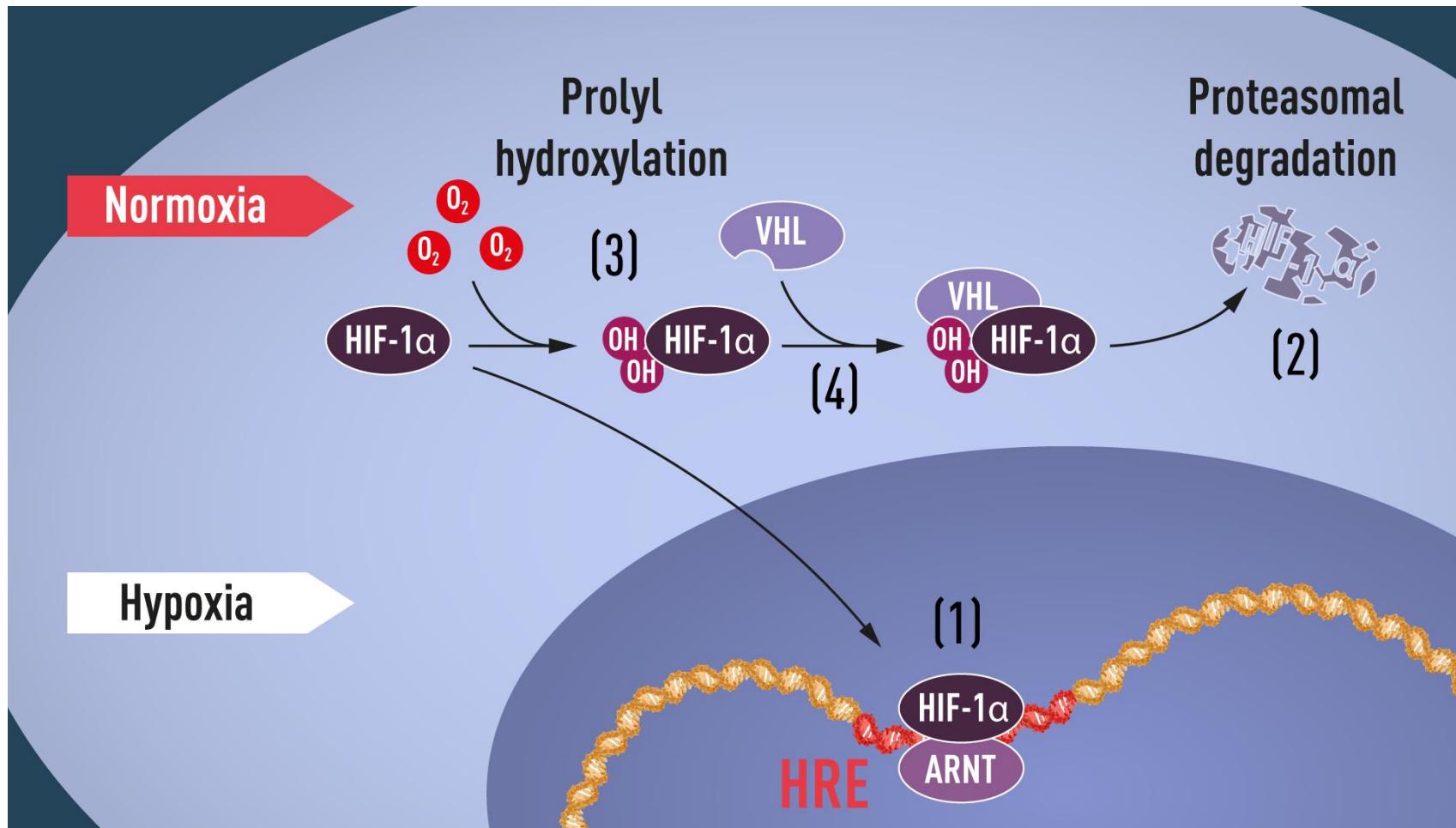
Normal O₂ → VHL binds HIF-1α → HIF-1α degradation

O₂ dependent binding of ubiquitin to HIF-1 α ?

Kaelin & Ratcliffe (2001)



How cells sense and adapt to oxygen availability?



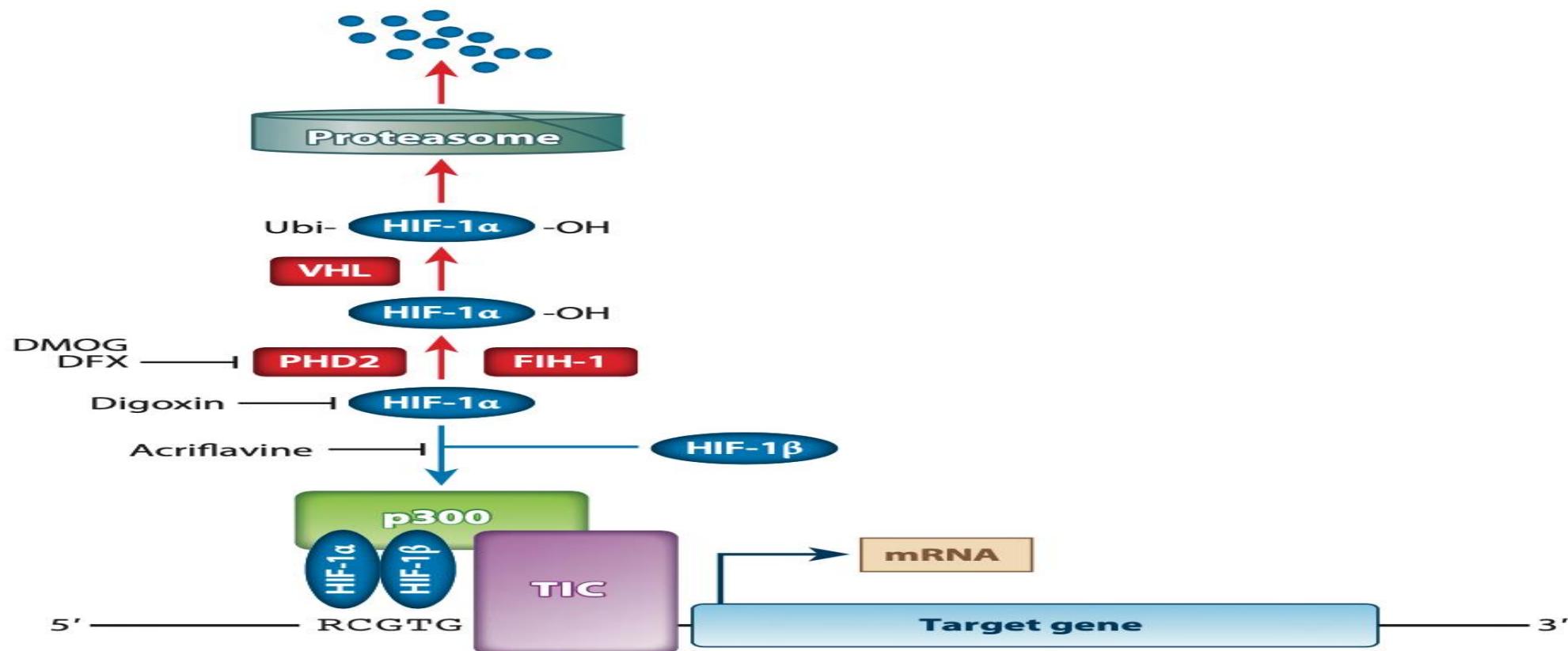
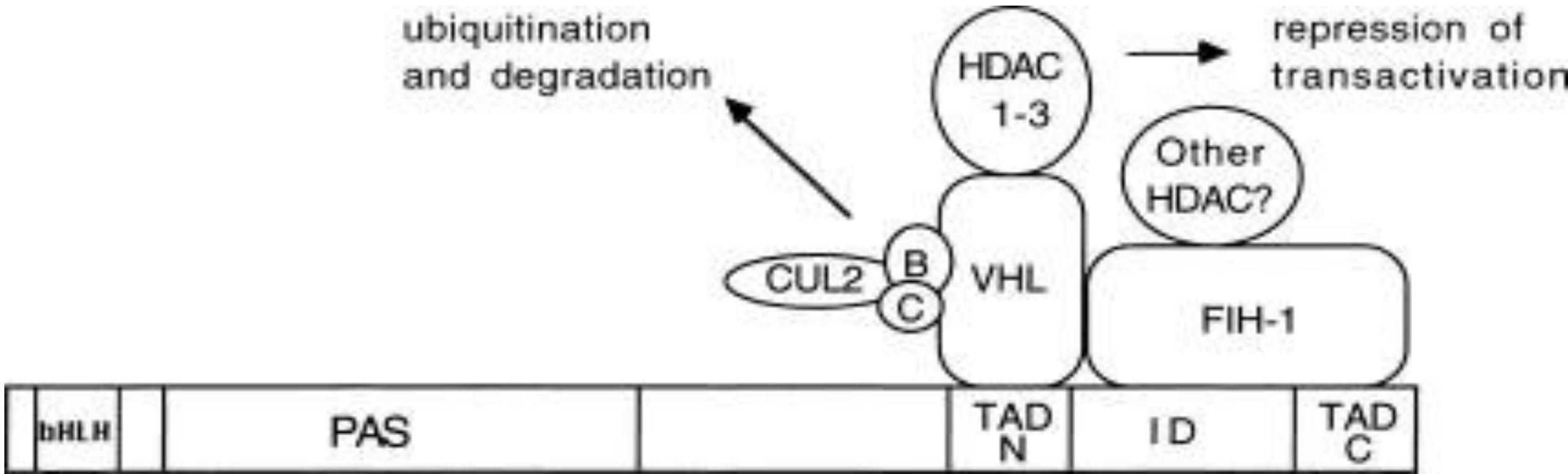
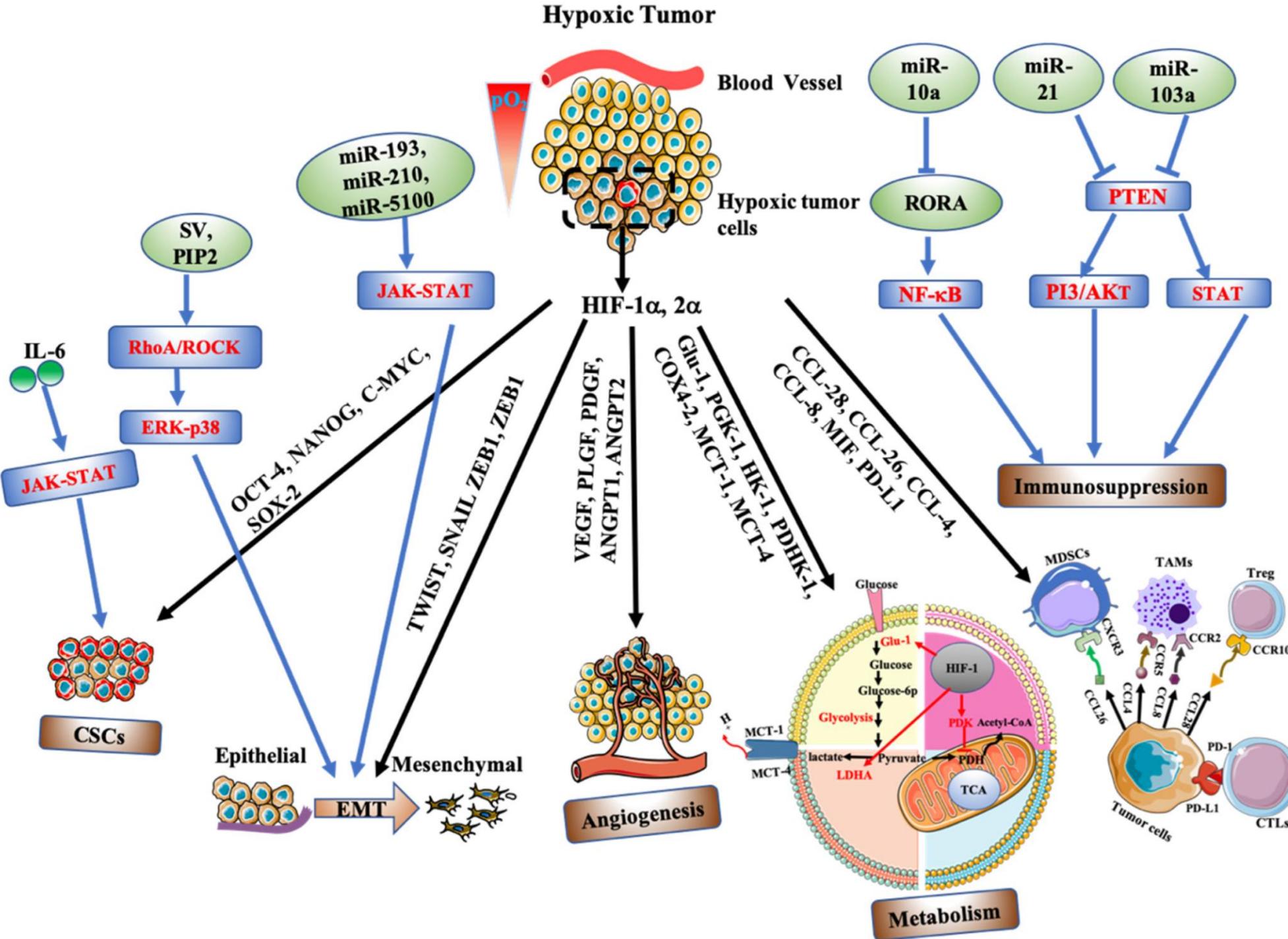


Figure 1

Hypoxia-inducible factor 1 (HIF-1) activates gene transcription in response to hypoxia. Under normoxic conditions (red arrows), HIF-1 α is subjected to hydroxylation on proline residues 402 and 564 by prolyl hydroxylase domain protein 2 (PHD2) and other prolyl hydroxylases. This hydroxylation is



Negative regulation of HIF-1 α protein stability and transcriptional activity under nonhypoxic conditions mediated by VHL and FIH-1. Elongins B and C and cullin 2 are required for E3 ubiquitin–protein ligase activity, whereas HDACs repress transactivation.



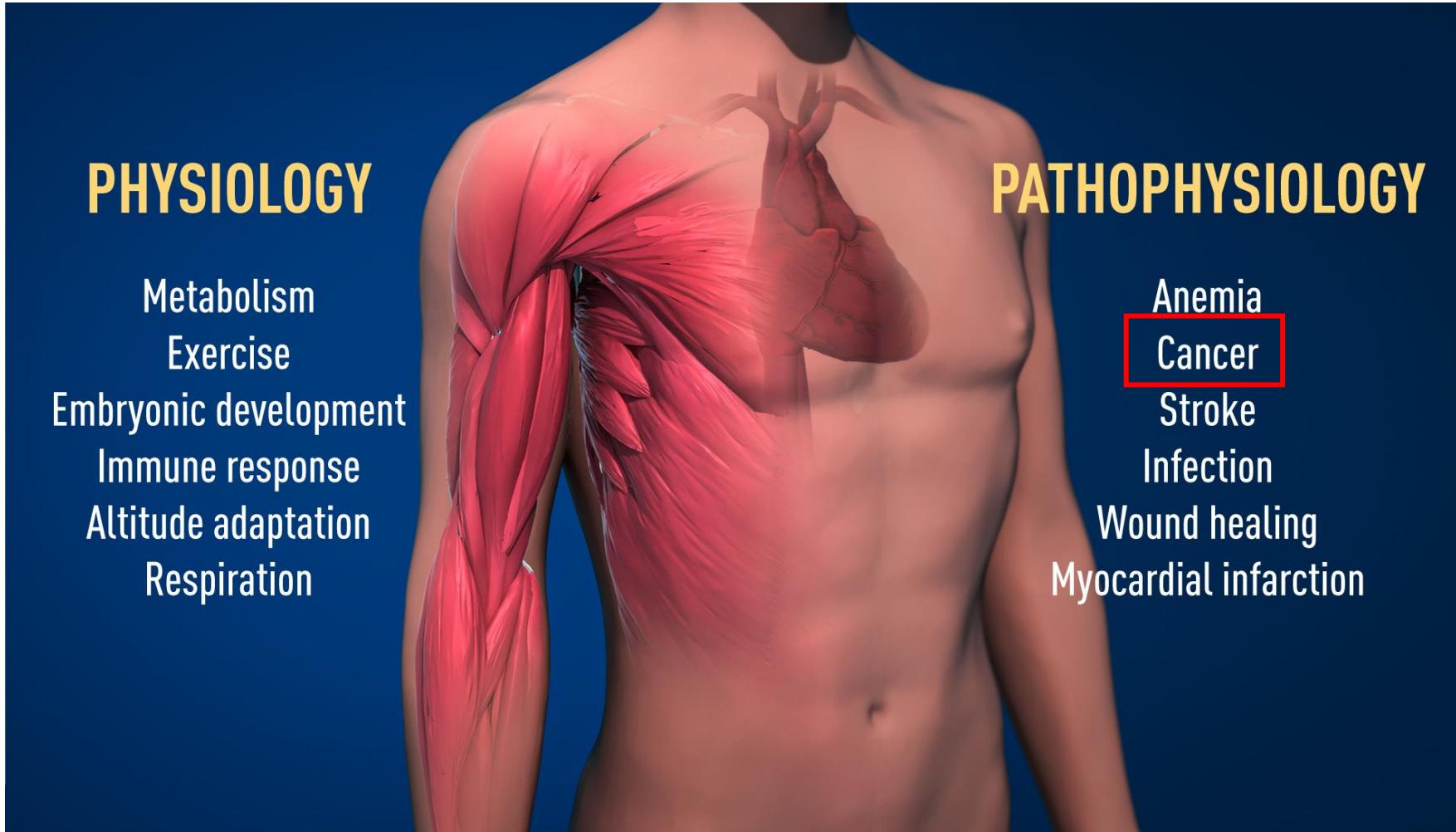
Silent hypoxia (deprivation of oxygen at the tissue level) has emerged as a major cause of death in COVID-19 patients

Usually, when your oxygen falls below 95%, you will have symptoms, breathlessness, a feeling of discomfort certainly. Now, in COVID-19, for some reason, that is not happening .

This is also called Happy hypoxia

The government plans to procure more pulse oximeters for the treatment of patients, especially those who are asymptomatic.

Why is O₂ sensing so important?



PHYSIOLOGY

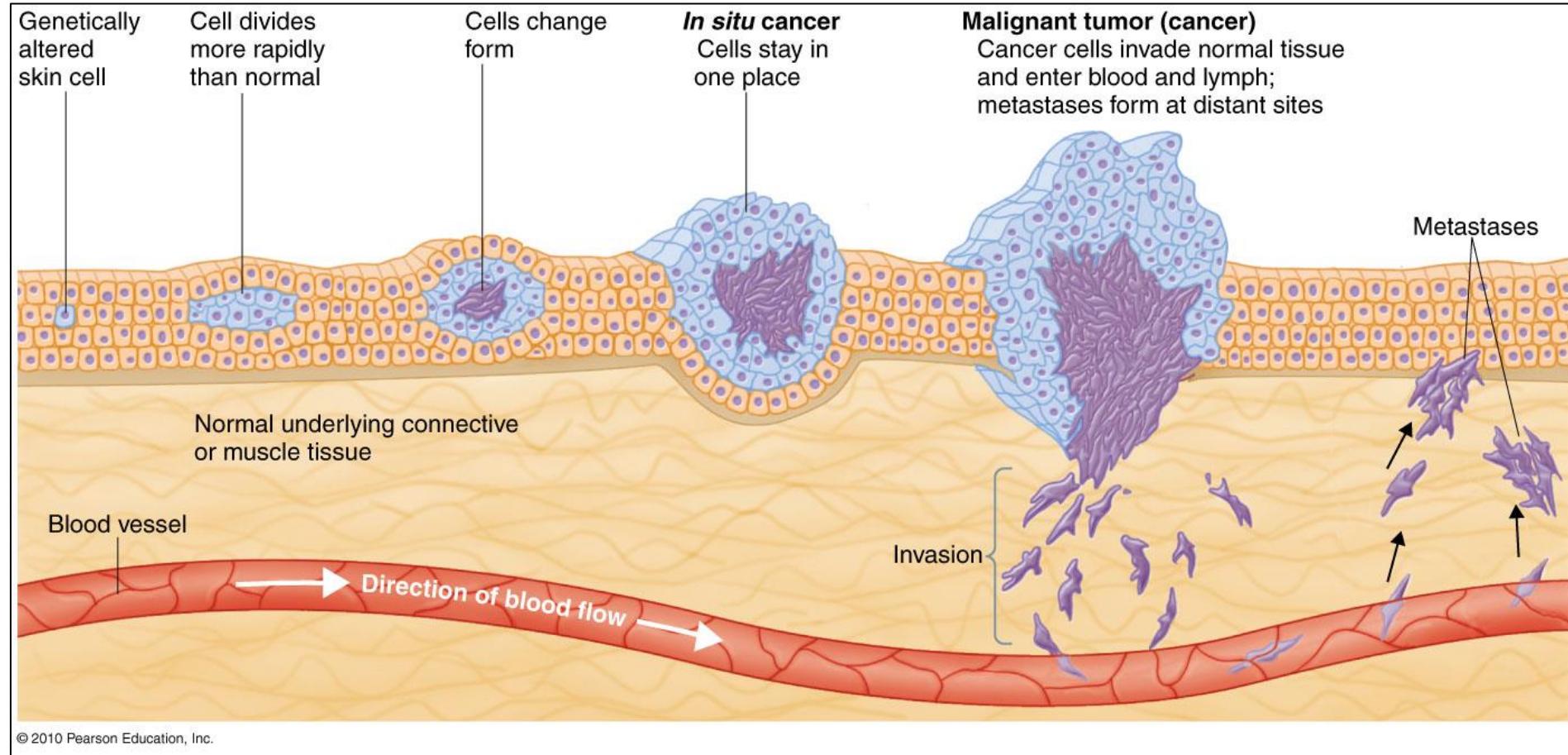
Metabolism
Exercise
Embryonic development
Immune response
Altitude adaptation
Respiration

PATHOPHYSIOLOGY

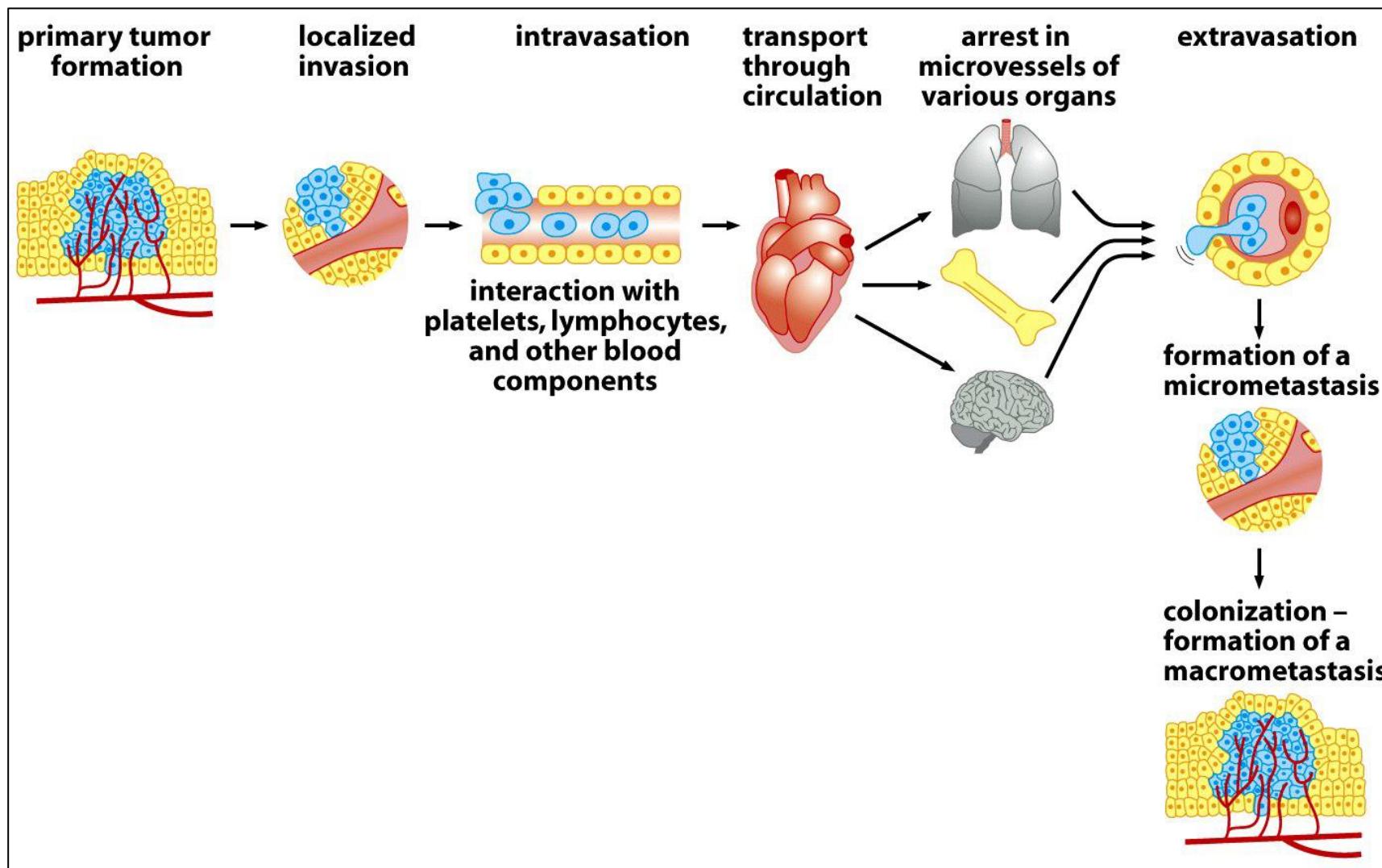
Anemia
Cancer
Stroke
Infection
Wound healing
Myocardial infarction

Cancer – Abnormal swelling in the body caused by uncontrolled cell proliferation and deregulated cell death caused by multiple changes in gene expression

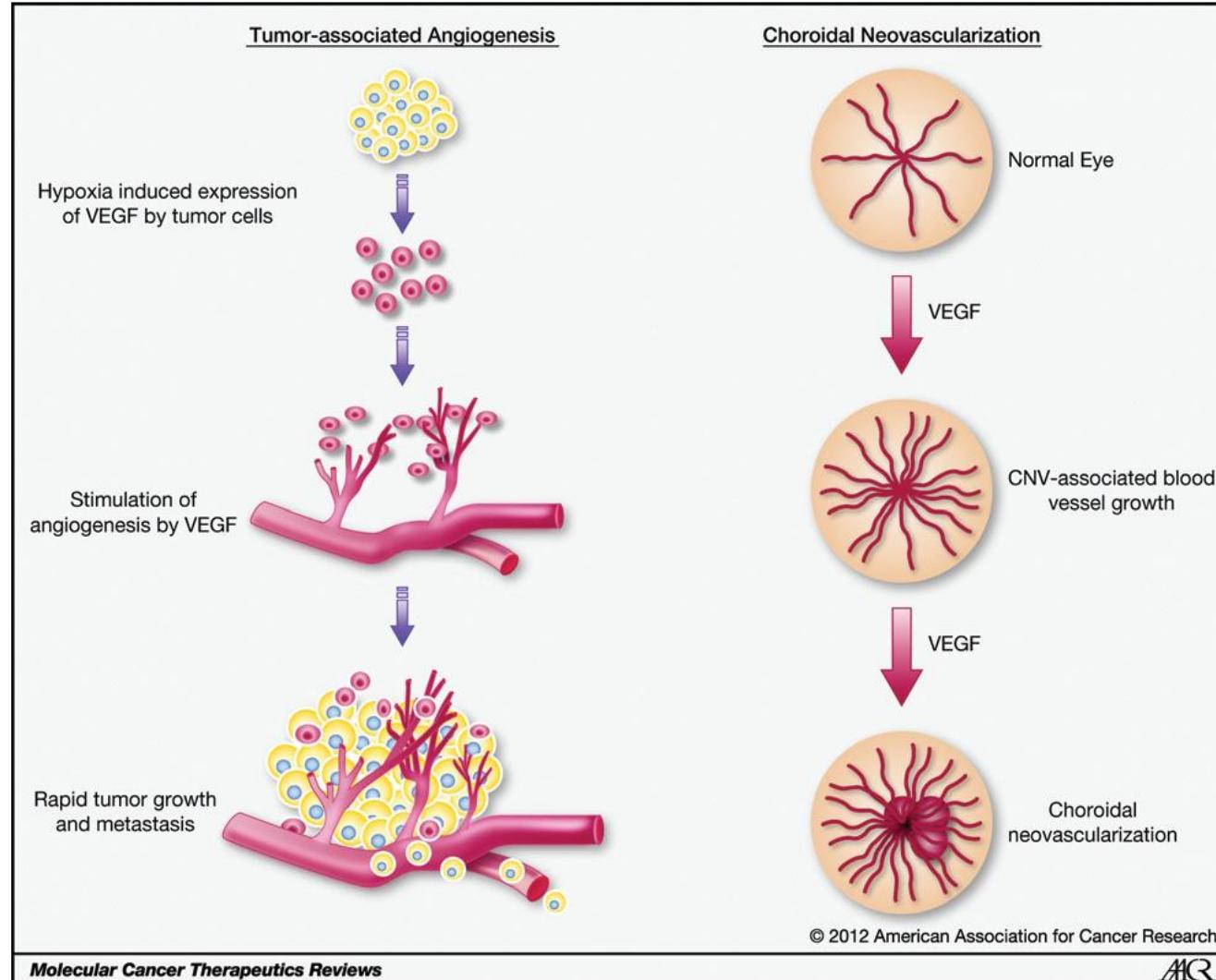
Development of a malignant tumor



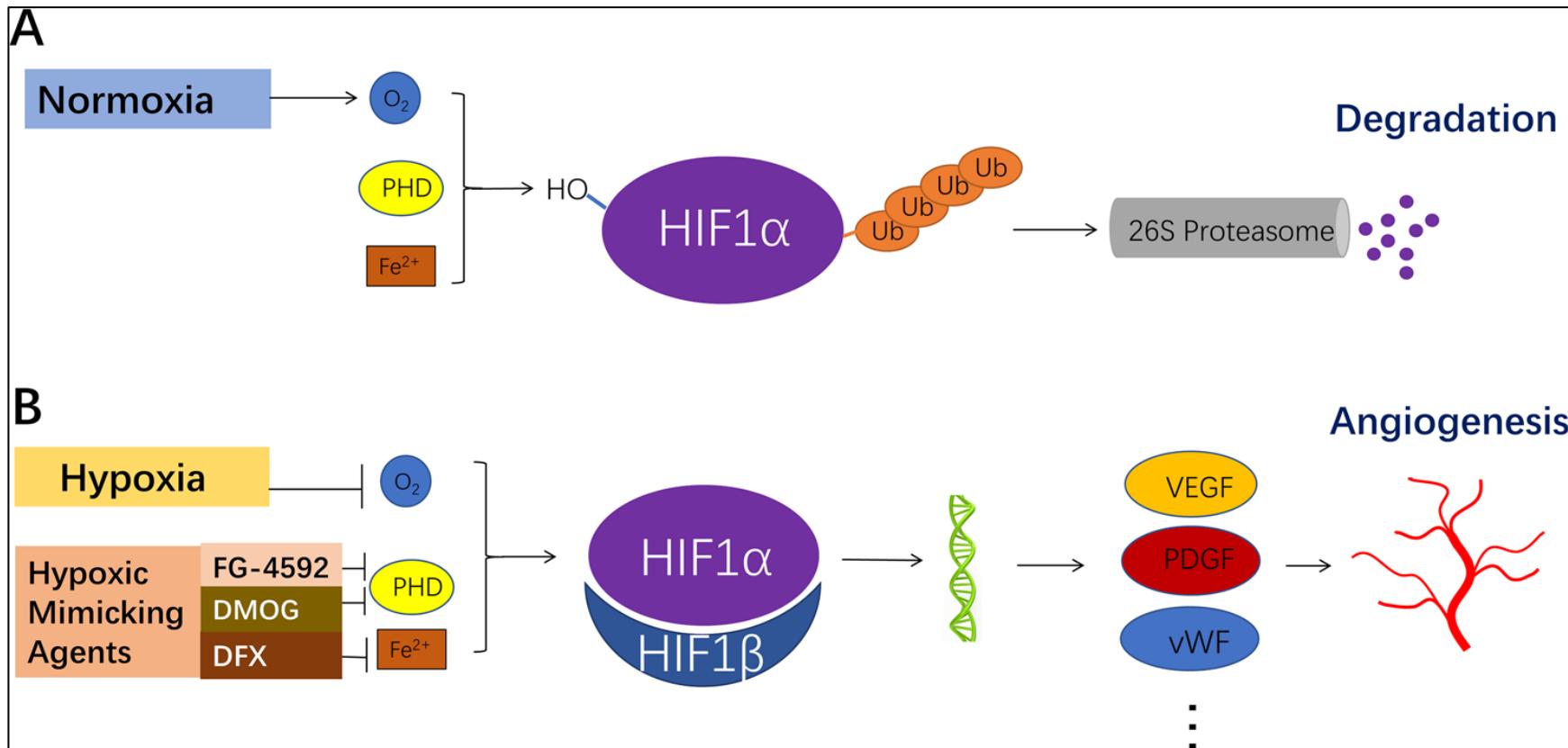
The Invasion-Metastasis Cascade



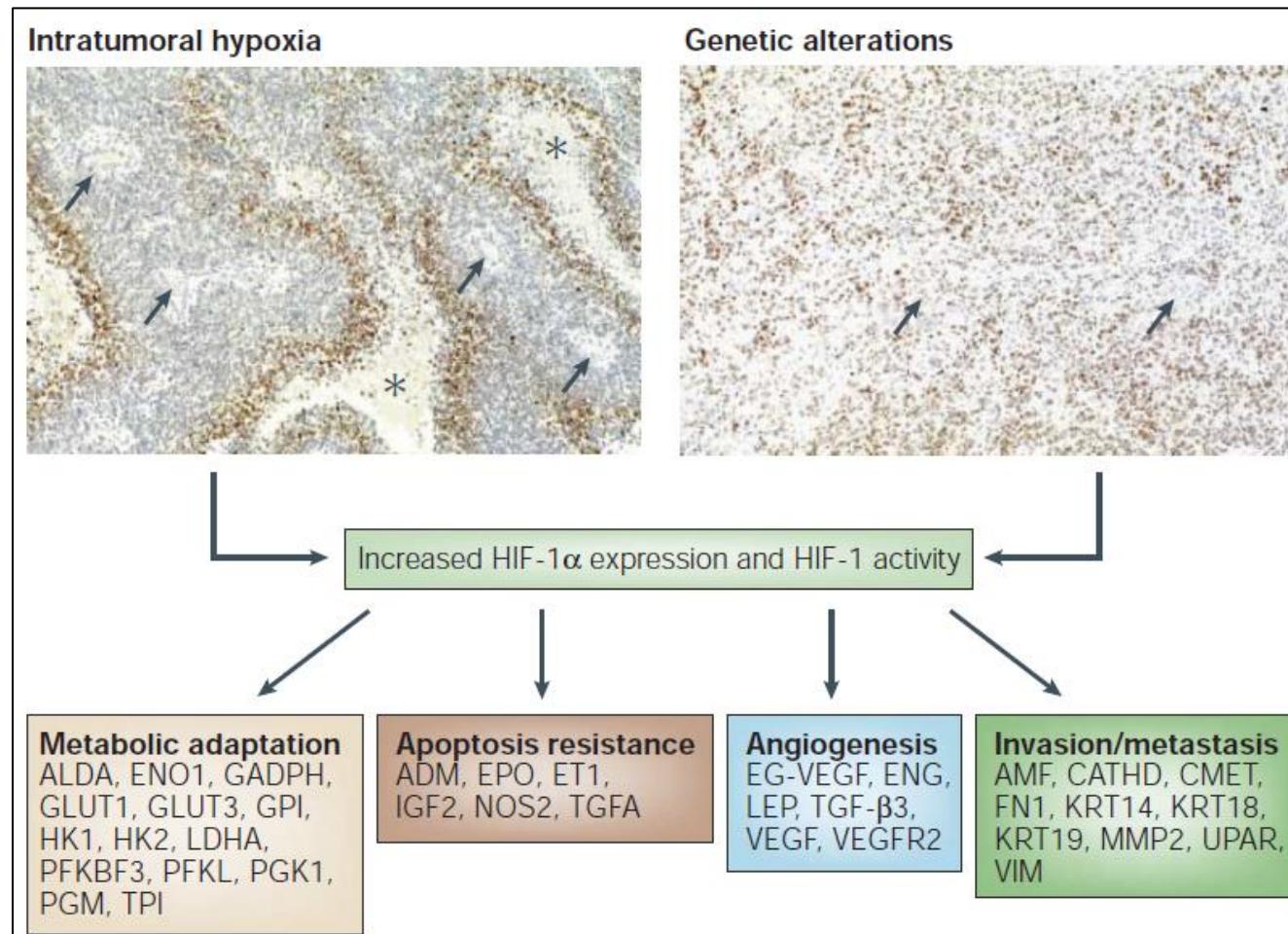
Schematic presentation of tumor-induced angiogenesis and choroidal neovascularization



Role of Hypoxia on HIF-1 α , VEGF and angiogenesis



Mechanisms and consequences of HIF-1 activity in cancer cells.



Involvement of HIF-1 in autocrine growth factor stimulation of cancer cells

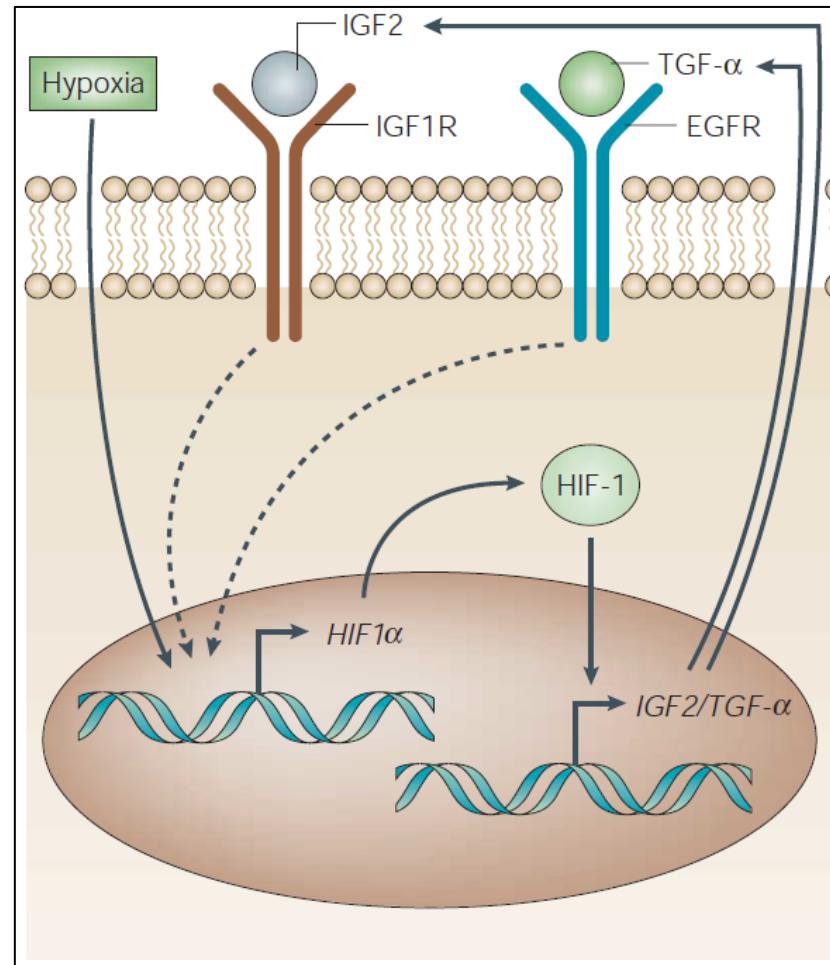


Table 1. Genetic alterations that increase HIF-1 activity

Alteration in tumour	Mechanism of HIF-1 α induction
VHL loss of function	Decreased ubiquitylation
p53 loss of function	Decreased ubiquitylation
PTEN loss of function	Increased synthesis
PI3K–AKT–mTOR signalling*	Increased synthesis
MEK–ERK signalling*	Increased synthesis
ERBB2 gain of function	Increased synthesis
EGFR signalling*	Increased synthesis
IGF1R signalling*	Increased synthesis
PGE ₂ signalling*	Increased synthesis
SRC gain of function	Increased synthesis
ARF loss of function	Decreased nucleolar sequestration
BCL2 overexpression	Not determined

*Increased signalling could be due to genetic alteration in a component of the pathway or an upstream activator. For example, AKT gain-of-function mutation or PTEN loss-of-function mutation induces PI3K–AKT–mTOR signalling; EGFR amplification or TGF- α overexpression induces EGFR (and PI3K–AKT–mTOR and MEK–ERK) signalling. EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinase; IGF1R, insulin-like growth-factor-1 receptor; MEK, MAP/ERK kinase; mTOR, mammalian target of rapamycin; PGE₂, prostaglandin E2; PI3K, phosphatidylinositol 3-kinase; VHL, von Hippel–Lindau protein.

Table 2. Increased HIF-1 α levels in human cancers

Tumour type	Association
Cervical, early stage	Increased mortality
Cervical, RTX	Increased mortality
Lung, NSCLC	Decreased mortality
Lung, NSCLC	Increased mortality (HIF-2 α)
Breast, LN-positive	Increased mortality
Breast, LN-negative	Increased mortality
Oligodendrogloma	Increased mortality
Oropharyngeal SCC	Increased mortality, radiation resistance
Ovarian	Increased mortality (with p53)
Oesophageal, early stage	Resistance to PDT (with BCL2)
Endometrial	Increased mortality
Head and neck SCC, S/P surgery	Decreased mortality
Head and neck SCC	Increased mortality (HIF-2 α)
GI stromal tumour of stomach	Increased mortality

*Protein levels determined by immunohistochemical analysis of biopsy samples. GI, gastrointestinal; LN, lymph node; NSCLC, non-small-cell lung cancer; PDT, photodynamic therapy; RTX, radiation therapy; SCC, squamous-cell carcinoma; S/P, status post.

Table 4. Novel therapeutic agents that inhibit HIF-1 activity

Agent(s)	Molecular target(s)
<i>Inhibitors of signal-transduction pathways</i>	
BAY 43-9006	RAF kinase
CCI-779	mTOR
Celebrex	COX2
PD98059	MEK
Trastuzumab (Herceptin)	ERBB2 receptor tyrosine kinase
ZD-1839 (Iressa), OSI-774	EGFR tyrosine kinase
Imatinib (Glivec)	BCR-ABL, PDGFR tyrosine kinases
<i>Small-molecule inhibitors of HIF-1 activity</i>	
2ME2	Microtubule polymerization
17-AAG	HSP90
Camptothecin, Topotecan	Topoisomerase I
Pleurotin, 1-methylpropyl 2-imidazolyl disulphide	Thioredoxin 1
YC-1	Not determined

BCR-ABL, breakpoint-cluster-region-Abelson-leukaemia; COX2, cyclooxygenase 2; EGFR, epidermal growth-factor receptor; HSP90, heat-shock protein 90; MEK, MAP/ERK kinase; mTOR, mammalian target of rapamycin; PDGFR, platelet-derived growth-factor receptor.

Hypoxia regulation of cancer metabolism

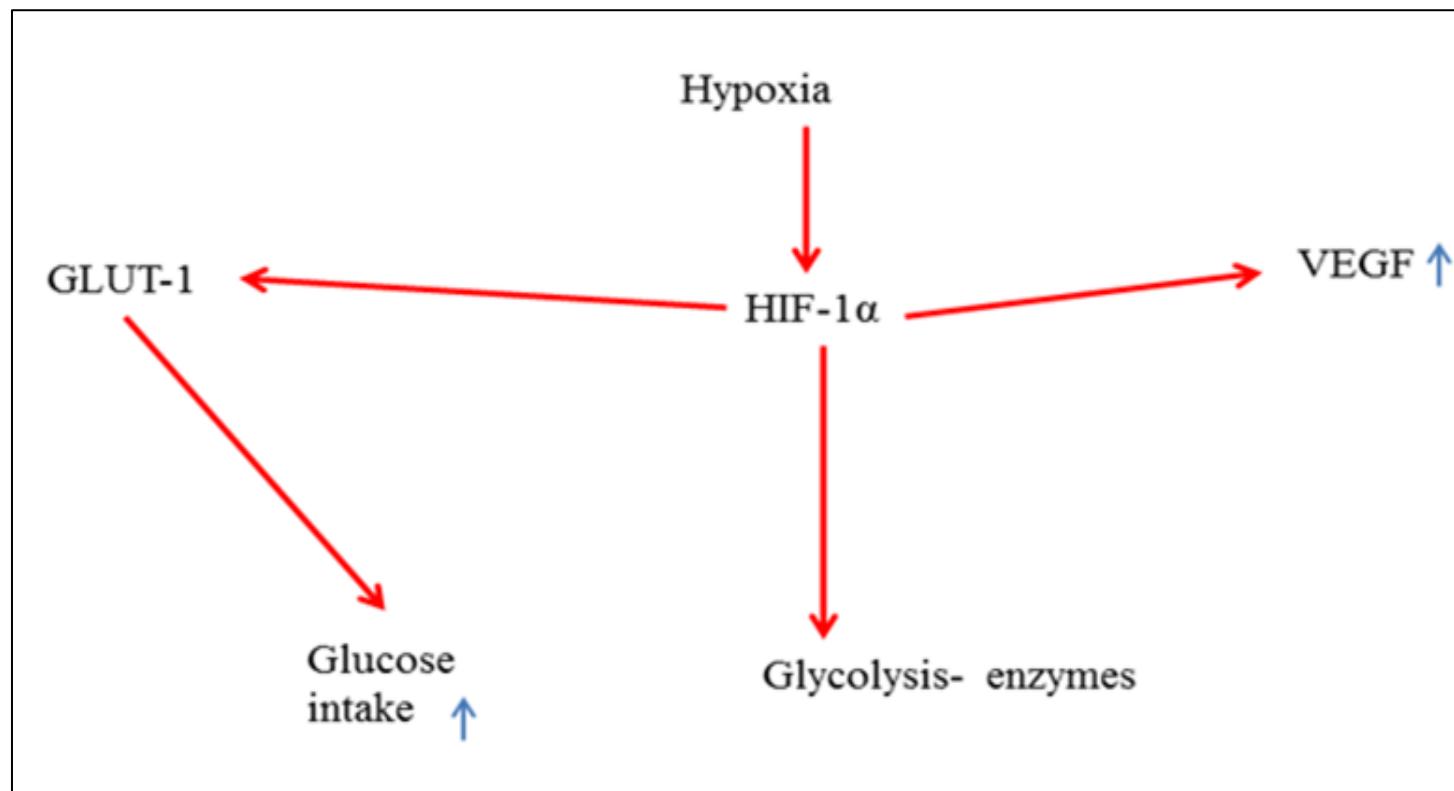
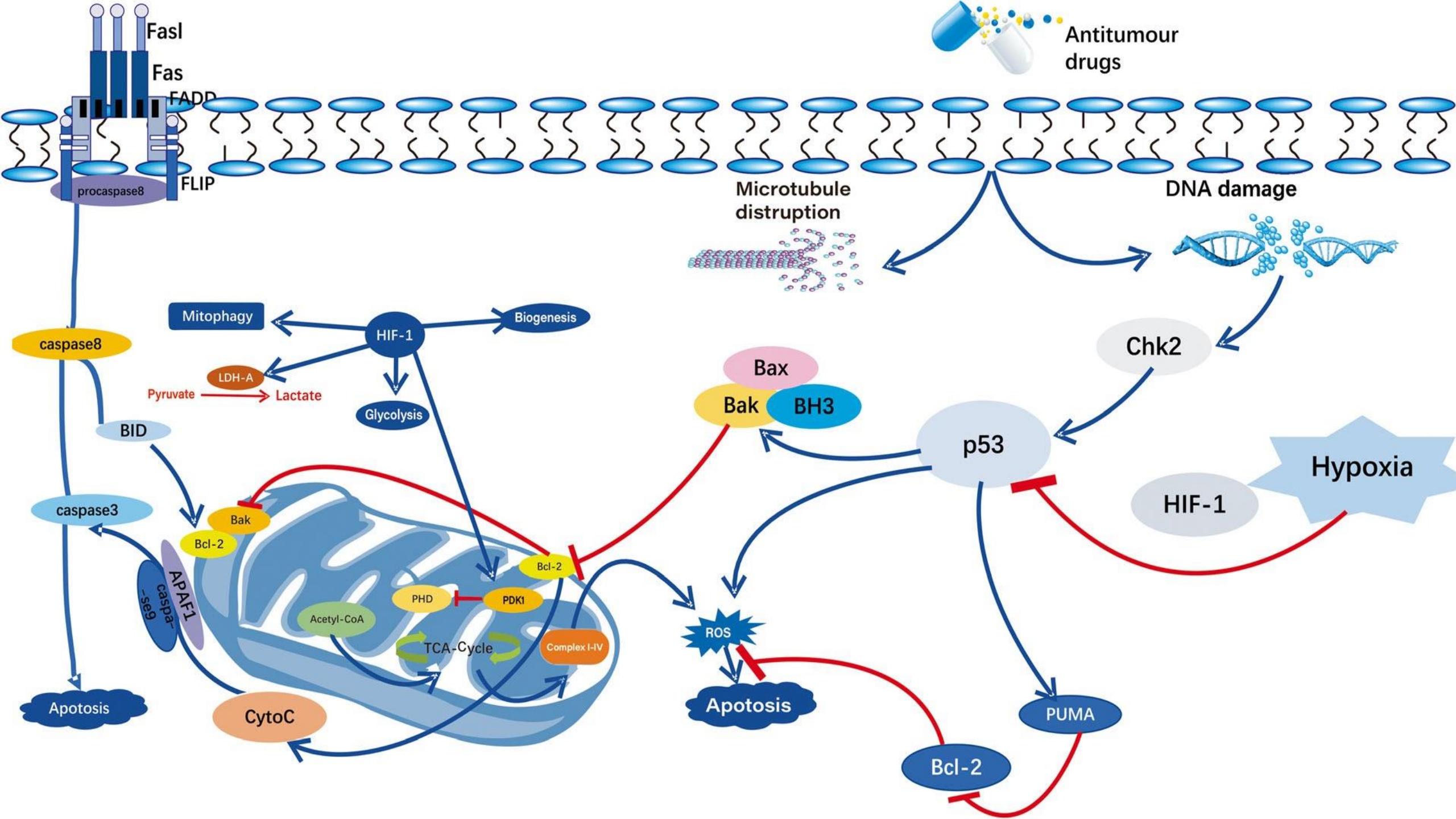


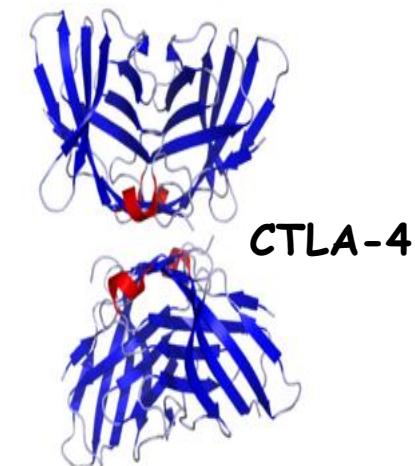
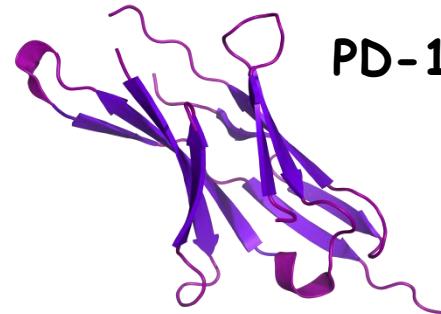
Table 5. HIF-1 in different cancer sites.

Site	Mechanism	Effect
BREAST	Increase cancer cell dormancy, blood vessels and expression of VEGF	Favoring osteolytic bone metastases and cancer spread
LUNG	Overexpression of VEGF, ROS production	High lymphatic invasion Low tumor differentiation Increased proliferation, poor prognosis
BONE	Inhibition of mesenchymal cell differentiation to osteoblast cell, increase of osteoclast differentiation through increase of IGF, VEGF, RANKL	Osteolysis, osteoblast apoptosis, inhibition of expression of osteocalcin
PROSTATE	Increased expression of growth factors and enzymes	Increased cell proliferation in O ₂ tension dependent-manner
COLORECTAL	Increase production of VEGF, COX2, MMP. Expression of ZEB gene	Increase of proliferation And spread of cancer cells, increase of EMT process



Cancer Therapy by Inhibition of Immune Checkpoints

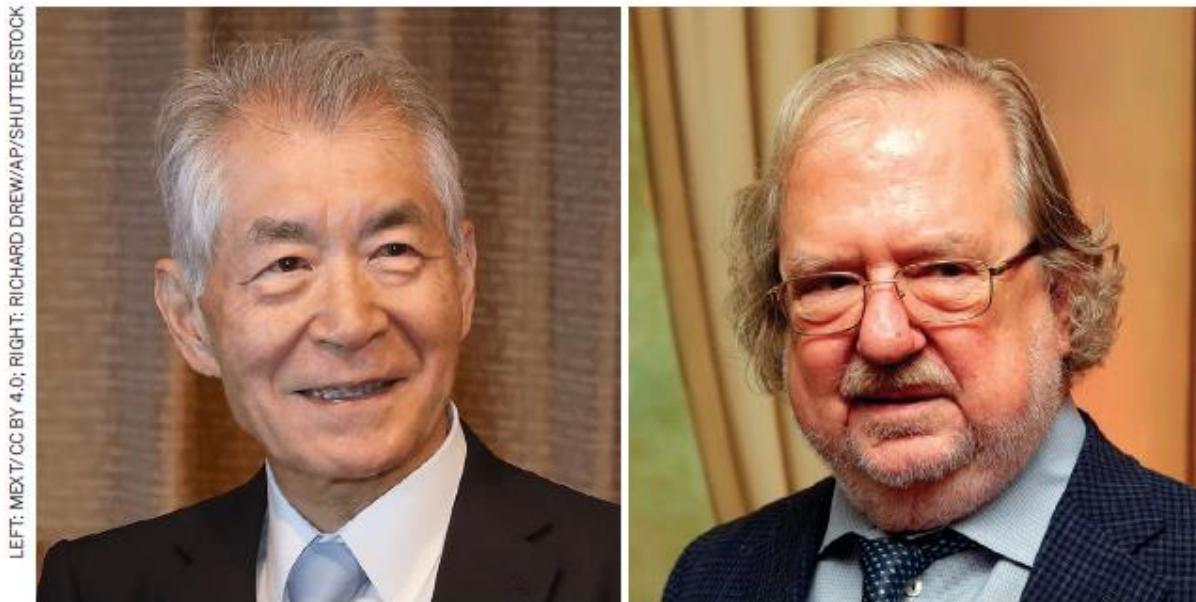
Programmed death-1
Cytotoxic T lymphocyte associated protein 4



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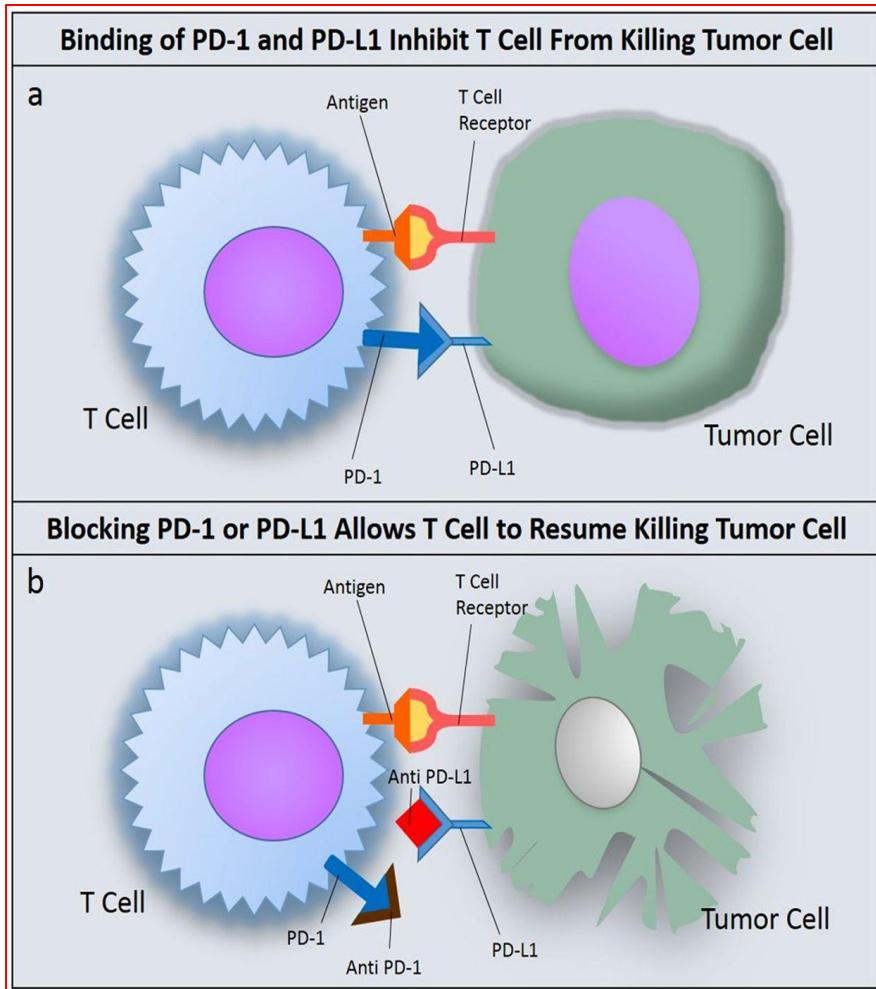
Discovery of checkpoint inhibitors

- **James Allison** at the University of Texas MD Anderson Cancer Center in Houston and **Tasuku Honjo** at Kyoto University in Japan have won the 2018 Nobel Prize in Physiology or Medicine.
- They showed how proteins on immune cells can be used to manipulate the immune system so that it attacks cancer cells.

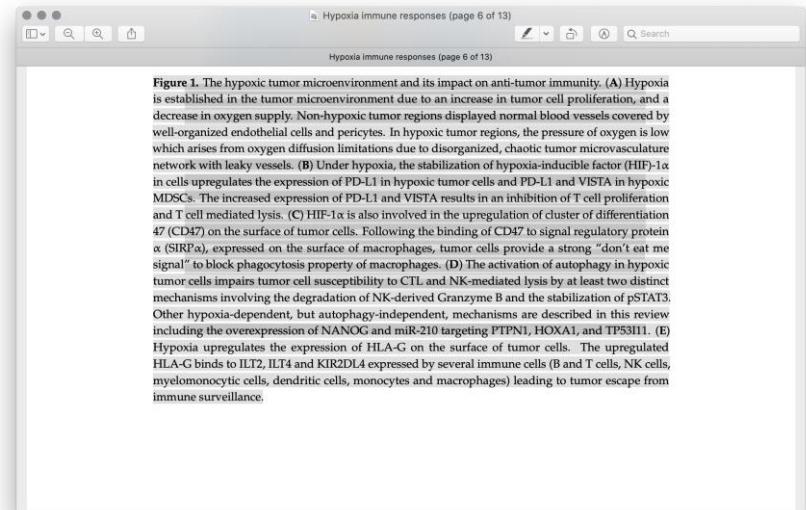
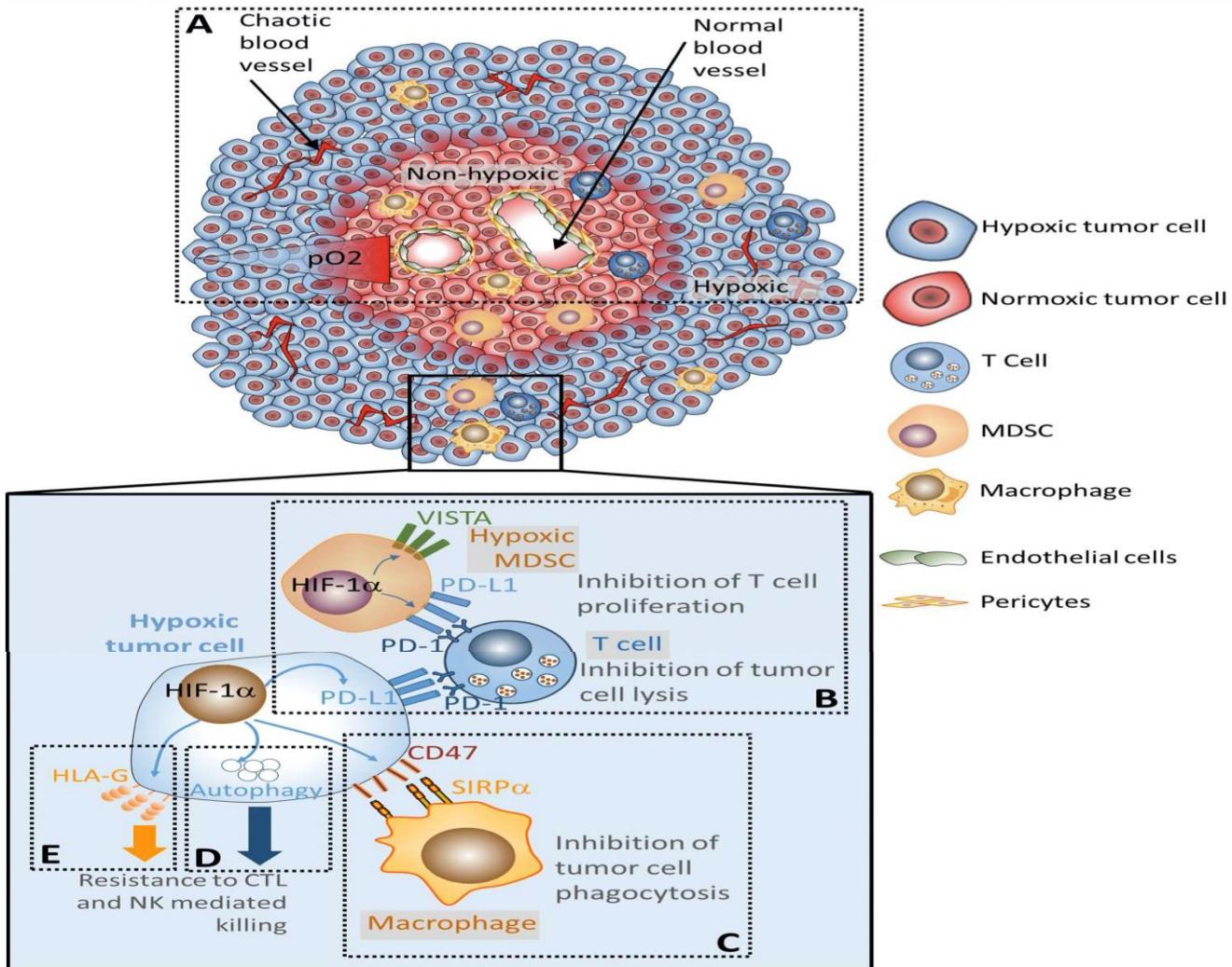


Tasuku Honjo (left) and James Allison share the 2018 Nobel Prize in Physiology or Medicine.

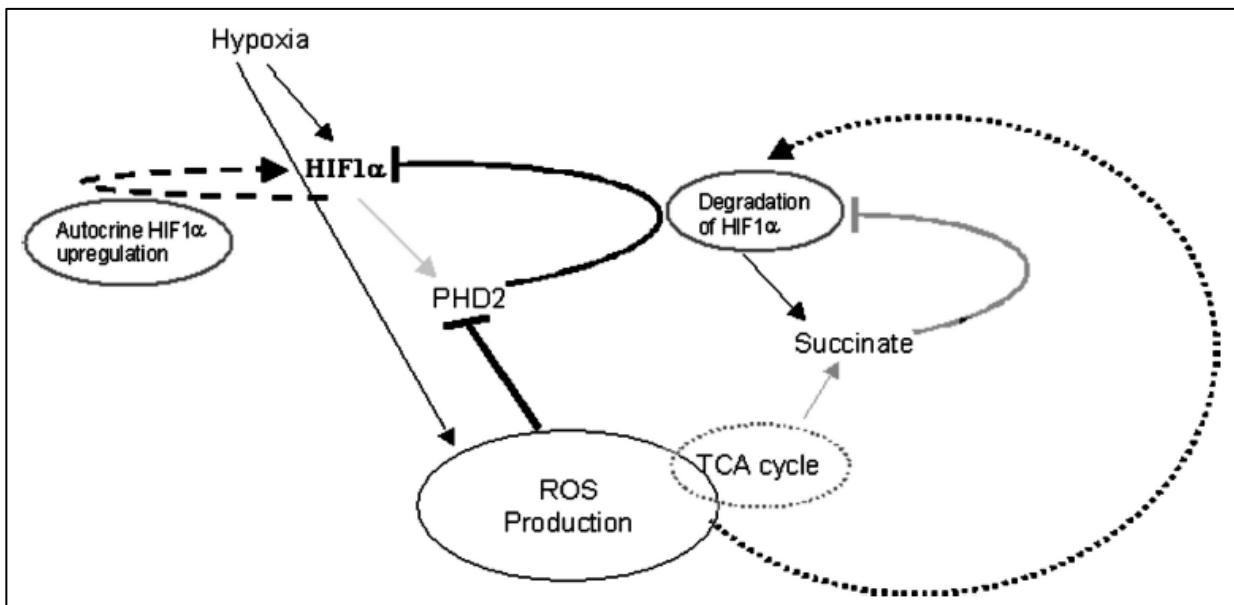
Tumors escape from host immune response



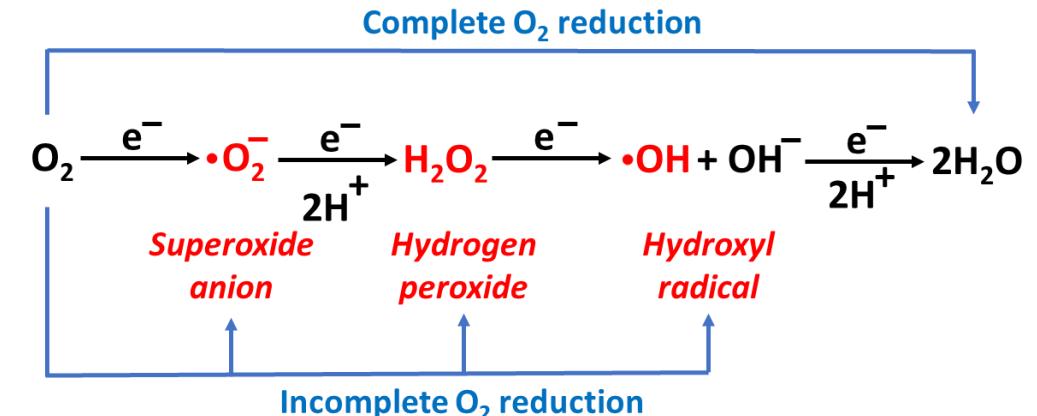
- Honjo demonstrated a novel mechanism
- Engagement of PD-1 by PD-L1 leads to the inhibition of Tcell proliferation and cytokine production.
- Tumors escape from the host immune response by expressing PD-L1 on their surface.
- This effect negatively regulates T-cell immune response through the interaction with PD-1, an immunoinhibitory receptor from CD28 family.



Schematic of the HIF1 system during chronic hypoxia and regulation of HIF-1 α by ROS



Generation of reactive oxygen species (ROS)



O_2 availability \downarrow \longrightarrow Intracellular ROS levels \uparrow

ROS production is due to the effects of hypoxia on the mitochondria electron transport chain (ETC)

Interestingly, hypoxia-driven ROS increase would then leave the mitochondria causing destabilization of Prolyl Hydroxylases (PHD) and stabilization of HIF1

ROS levels in normal versus cancer cells

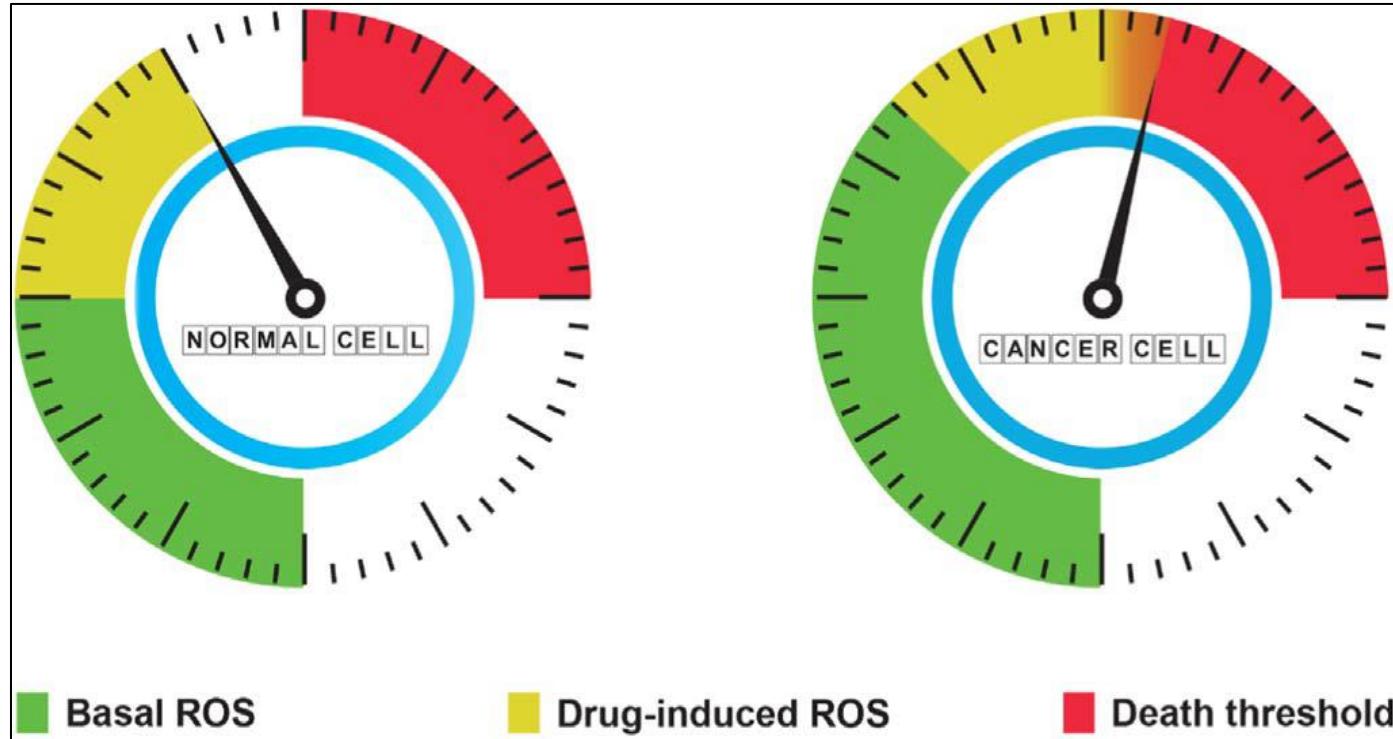
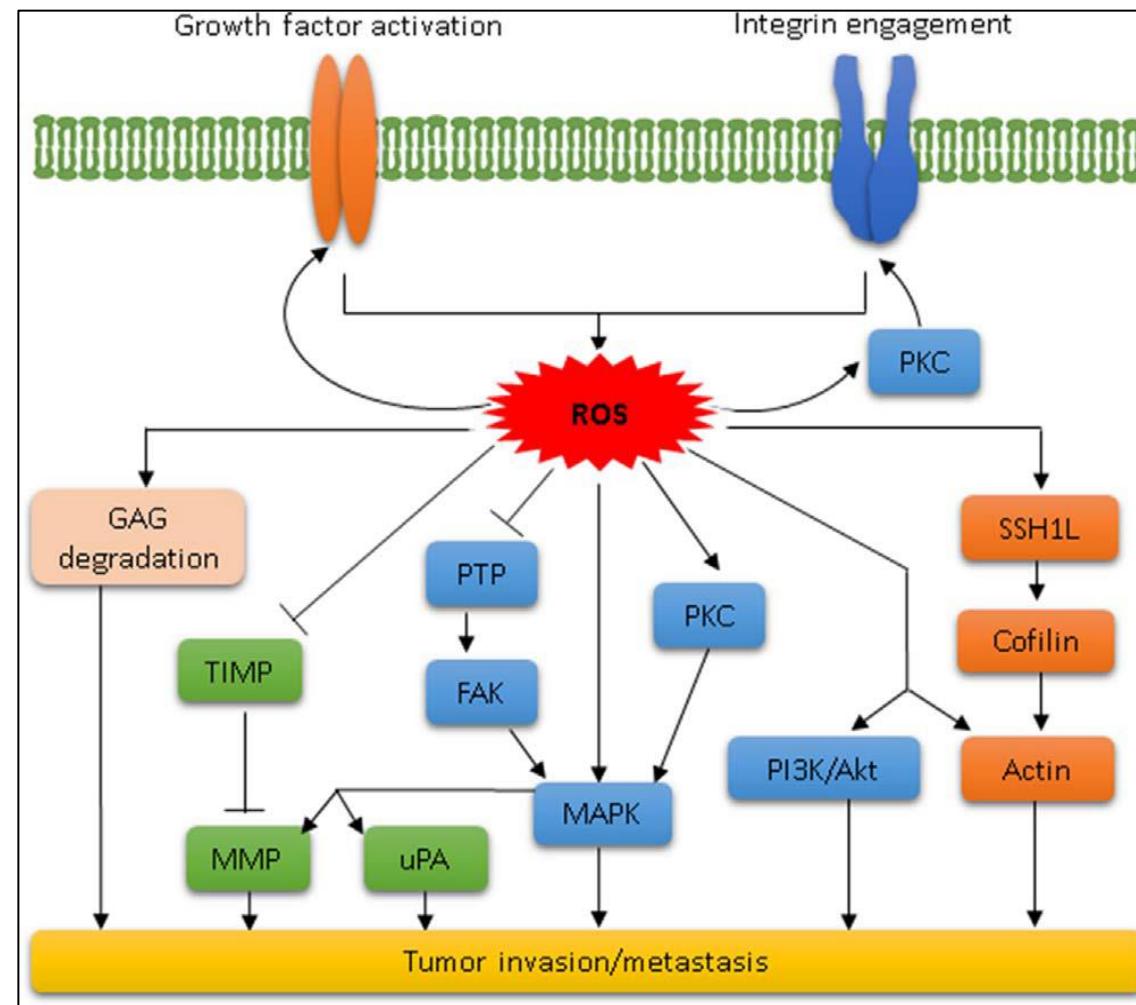


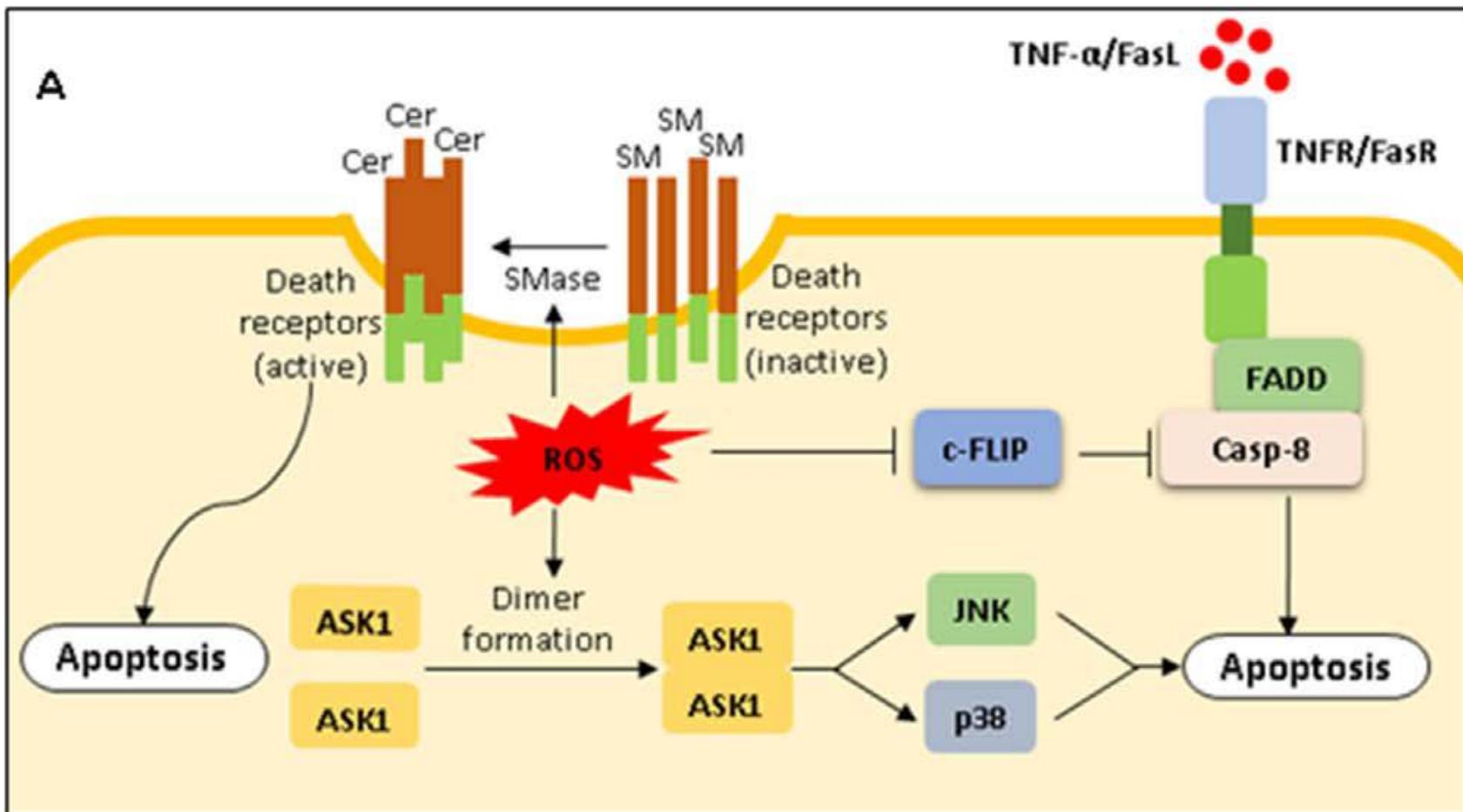
Table 6. Regulation of MiRNAs by HIF-1 α (a ROS-sensitive transcription factor) mediates metastasis

Transcription factors (TFs)	Activity of TFs changed by ROS	Target miRNAs	Cancer types	Outcomes of miRNA upregulation
HIF-1 α	↑	miR-210	PSCC PCa	↓ angiogenesis ↓ proliferation
HIF-1 α	↑	miR-382	gastric cancer	↑ angiogenesis ↑ proliferation
HIF-1 α	↑	miR-421	gastric cancer	↑ metastasis ↑ chemoresistance
HIF-1 α	↑	miR-191	Breast cancer	↑ metastasis

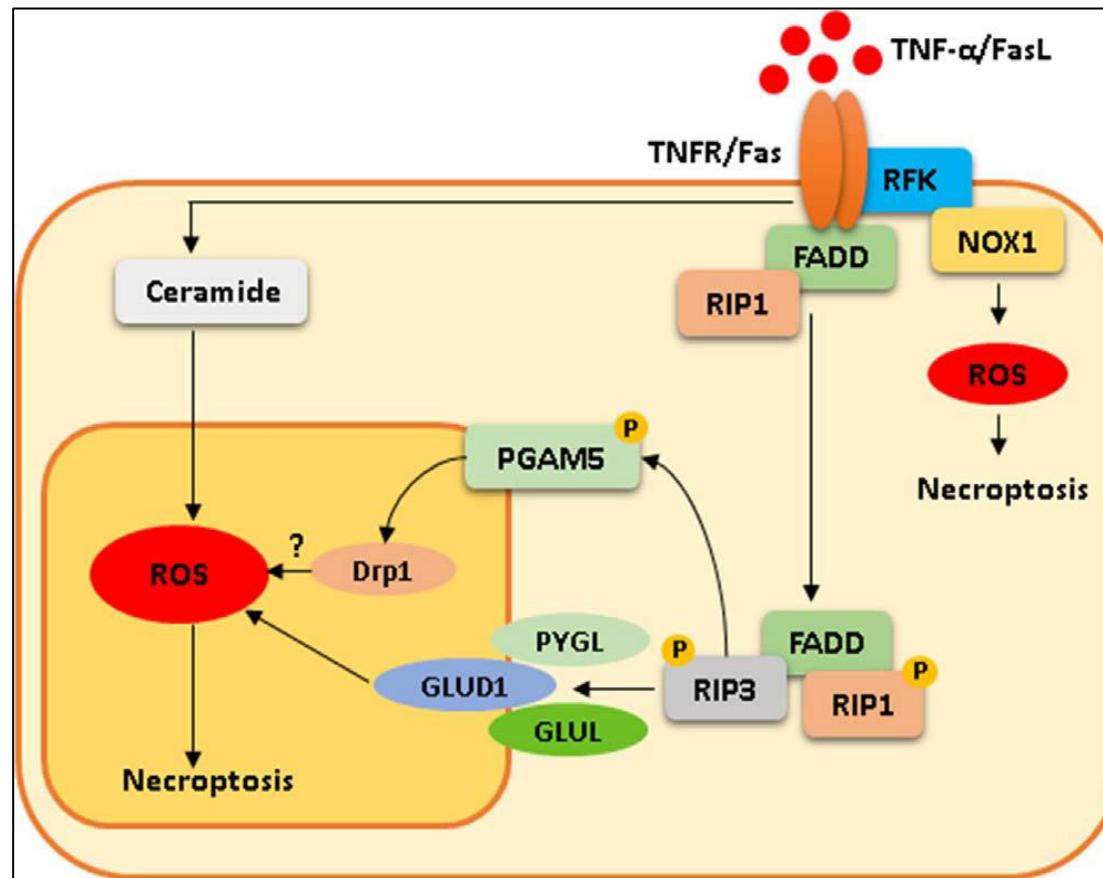
ROS regulation of invasion/metastasis



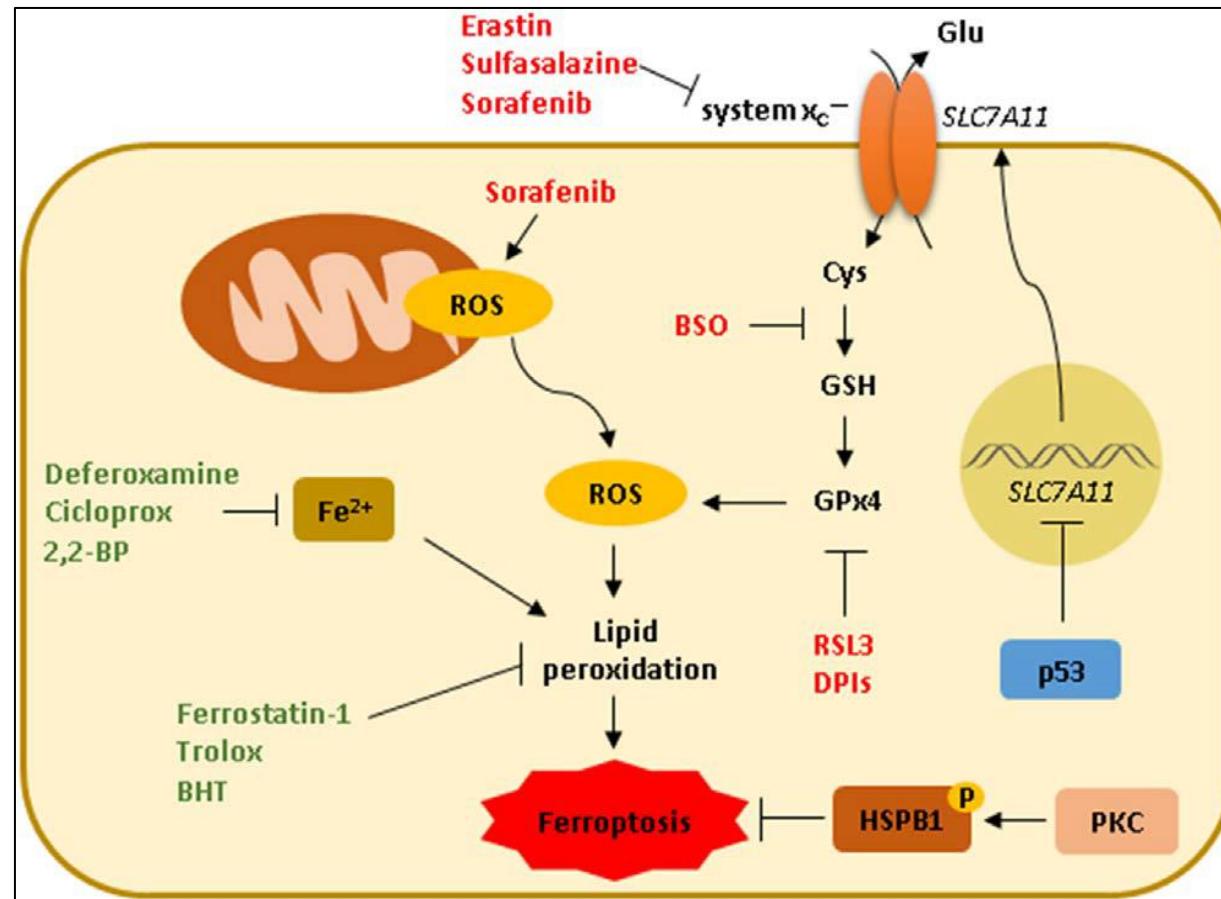
Pathways of ROS-driven apoptotic responses



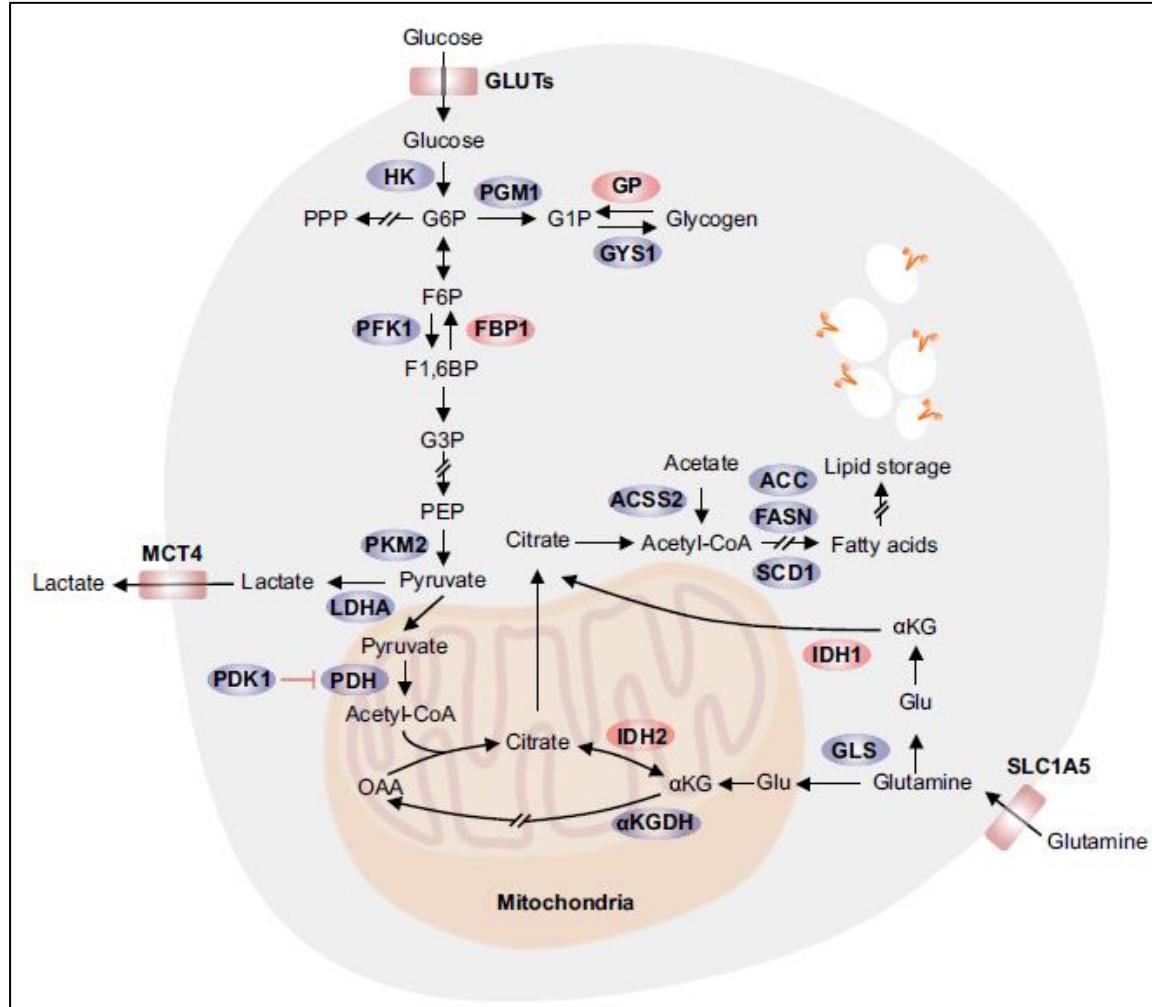
Pathways of ROS-driven necroptotic responses

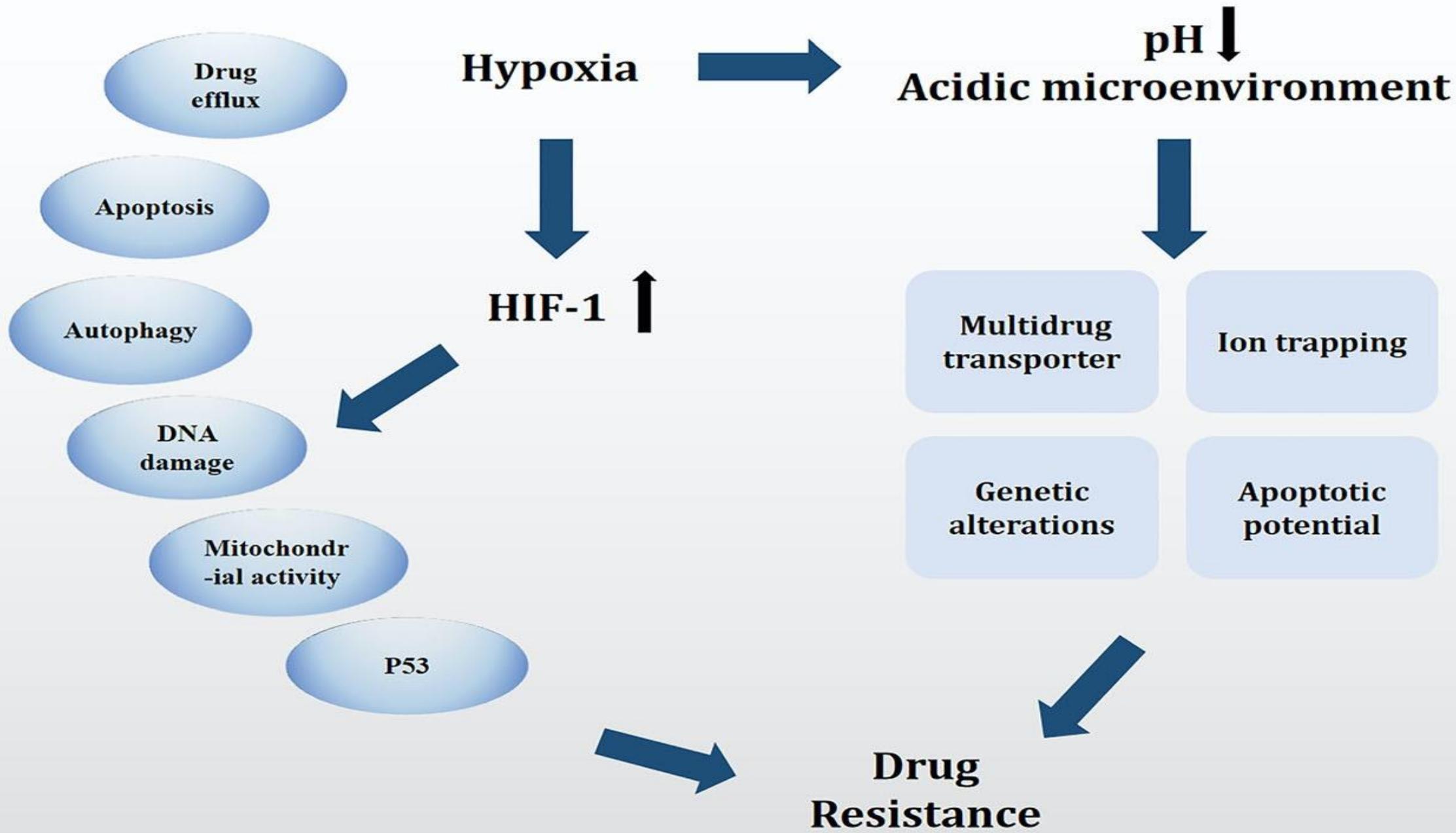


Pathways of ferroptosis regulation

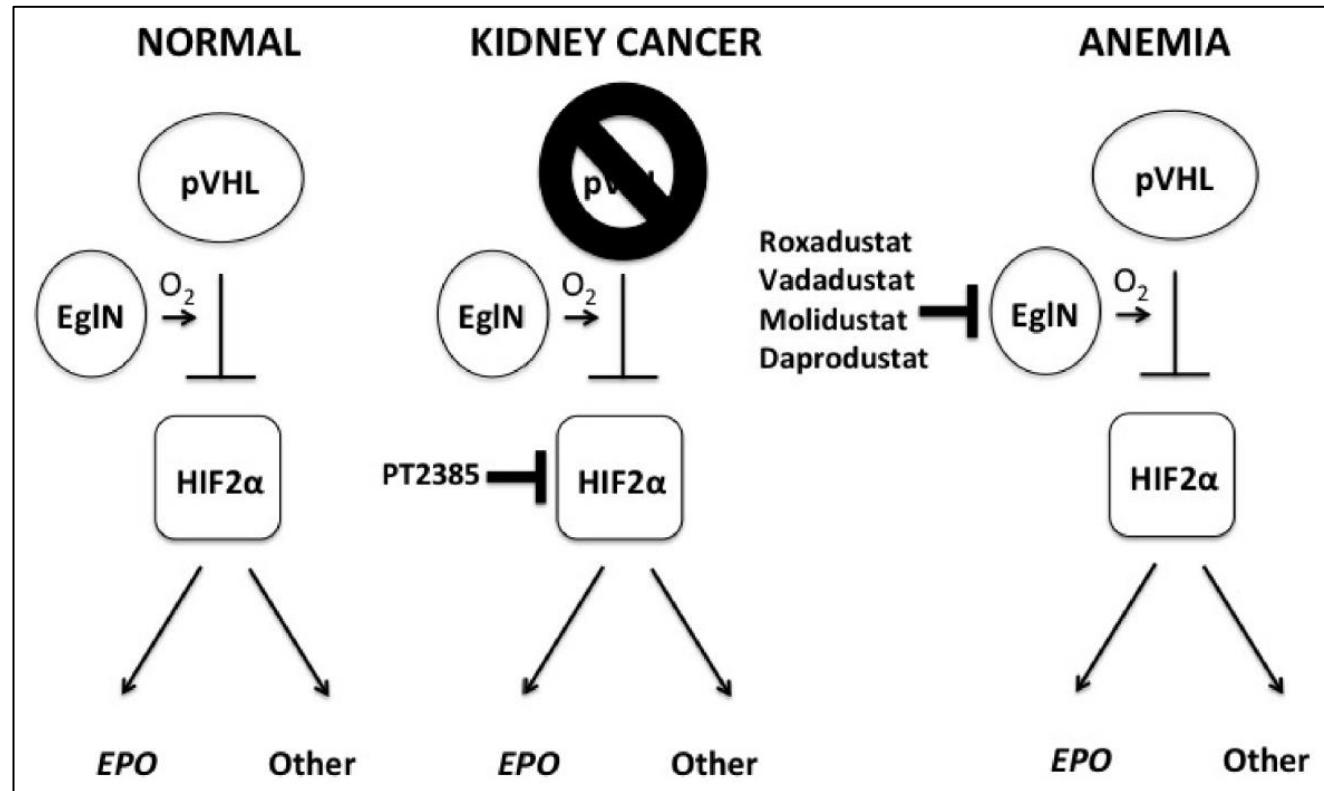


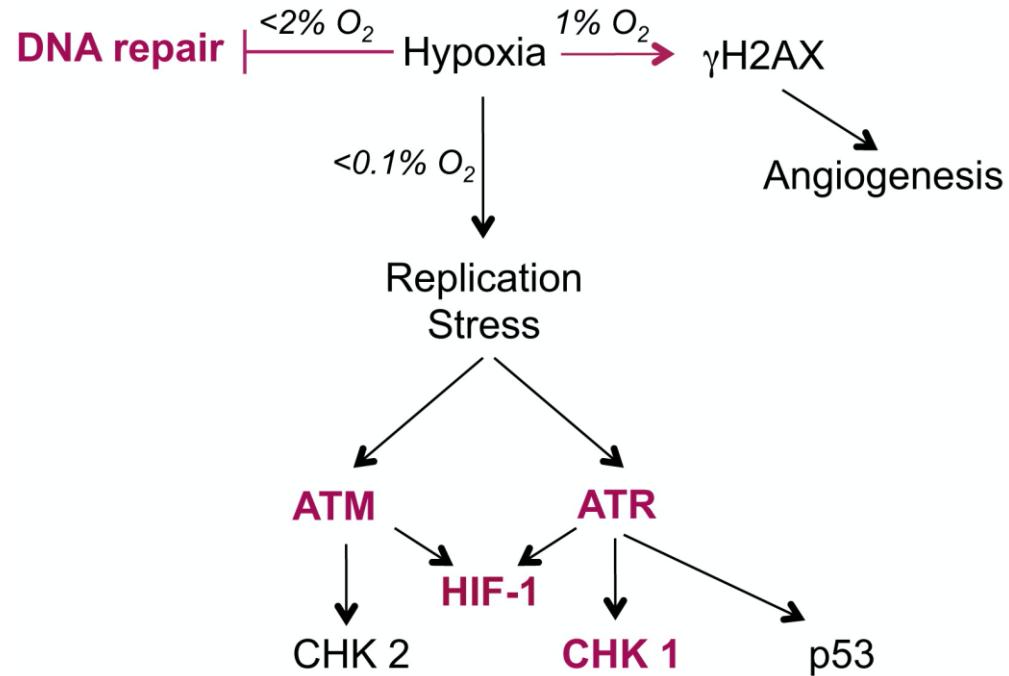
Metabolic reprogramming in cancer cells under hypoxia





Pharmacological manipulation of the oxygen-sensing pathway

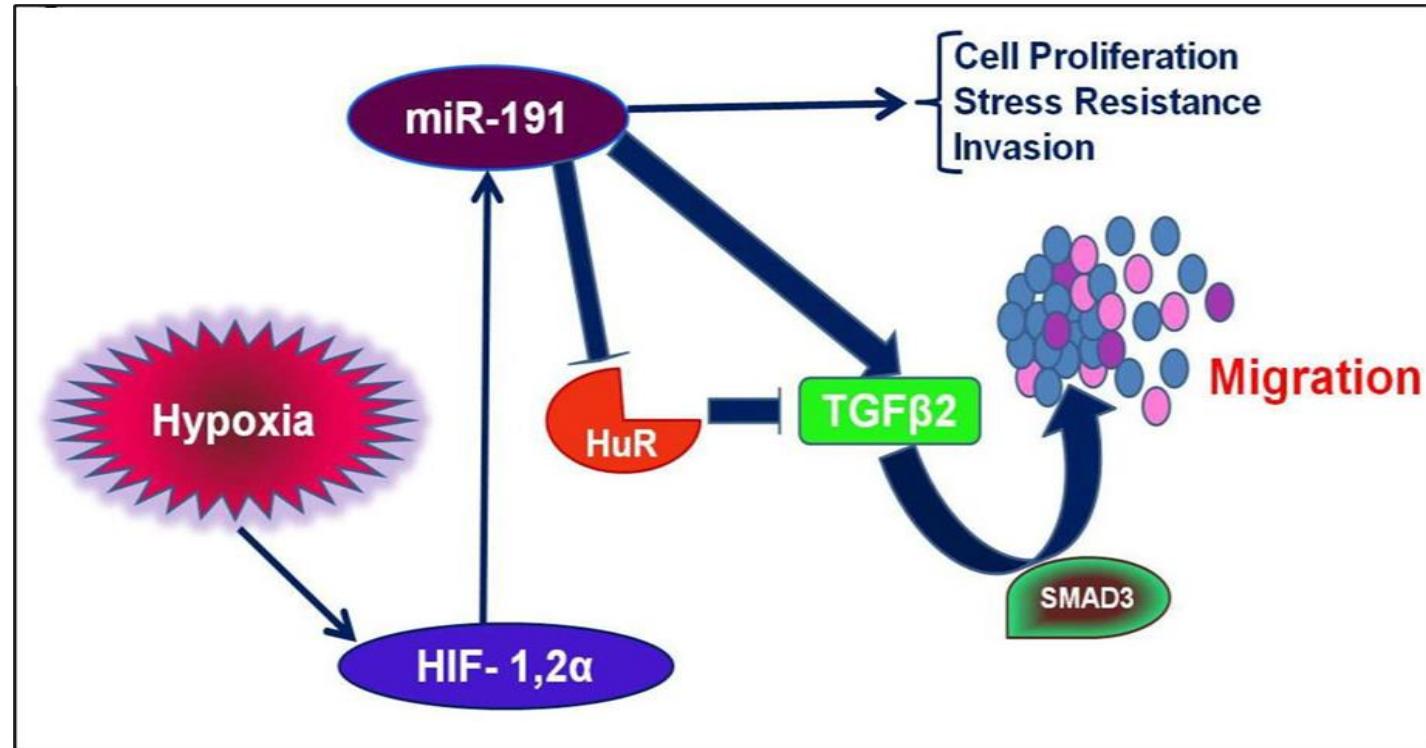




Homologous recombination, mismatch repair and non-homologous end-joining have all been shown to be less effective under hypoxic conditions, suggesting that a general response to hypoxia is repression of DNA repair

The mechanisms of DNA repair gene/protein repression are varied and include roles for HIF, micro-RNAs and epigenetic modifications

HIF-induced breast cancer migration under hypoxia is mediated by TGF β 2 and miR-191



7th International conference Translational Cancer Research

Feb 08 – 11, 2018, Westin Hotel, Chennai, India



Thank you . . .