

KSC2015 (대한심장학회 제59차 추계학술대회)

Lactate-Induced Cell Signaling in Hypoxic Microenvironment

2015. 10. 17.

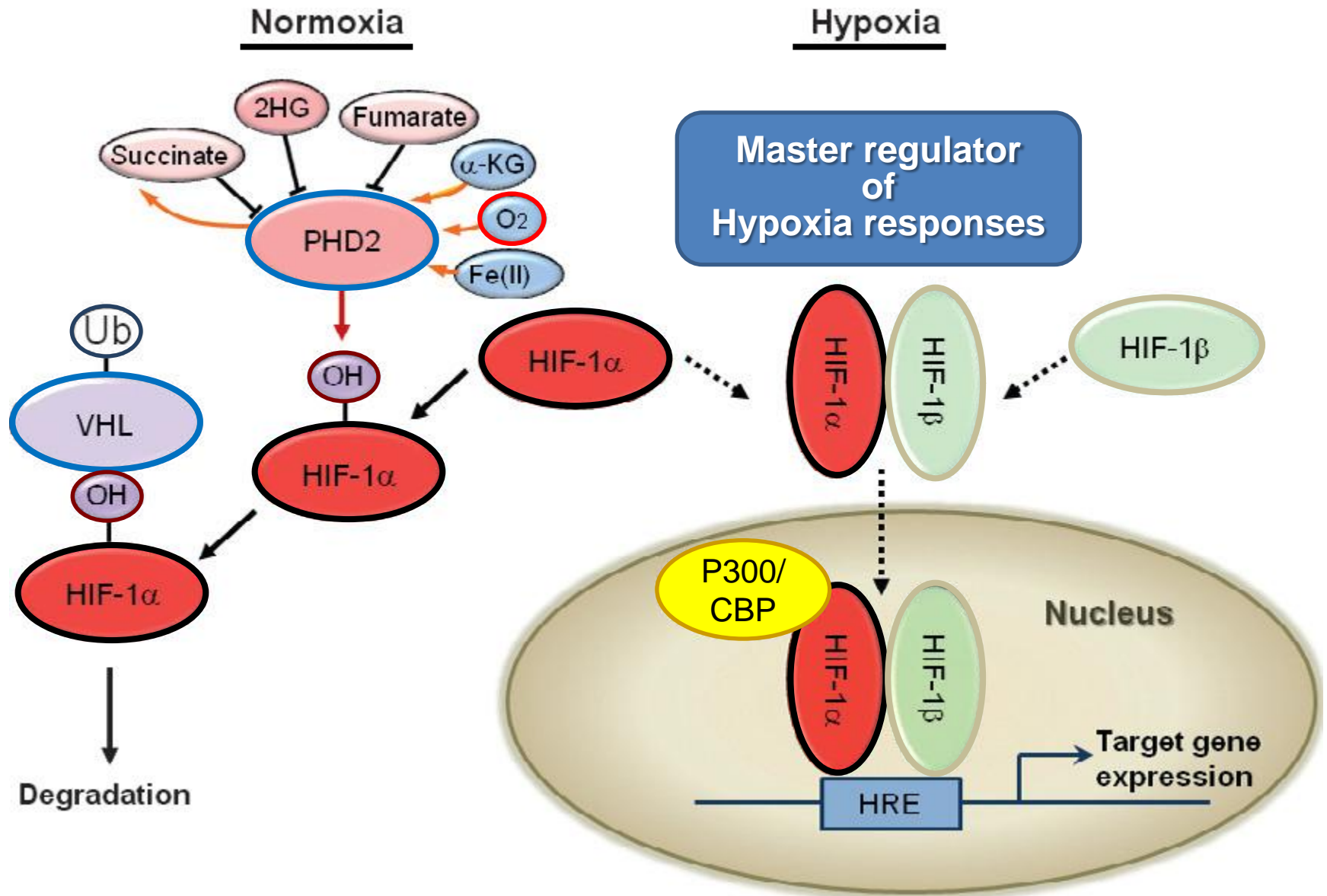
Korea Research Institute of Bioscience & Biotechnology
(KRIBB)

YEOM YOUNG IL

Hypoxia

- A condition in which the body or a region of the body is deprived of adequate oxygen supply
- Effects of hypoxia on cell physiology
 - Shrinkage of cellular activity and vitality
 - Prolonged hypoxia → Cell death
- **Hypoxia responses**
 - Cellular responses to prolonged hypoxia associated with a venous blood oxygen level below 6% (40mmHg)
 - Two types of responses
 - **Increasing the oxygen supplying capacity**; Angiogenesis, Erythropoiesis
 - **Maintenance of cell viability and growth**; **Metabolic reprogramming**, Defending cell death, Promoting hypoxic cell growth, Maintaining acid-base balance, Increasing cell motility

HIF-dependent regulation of hypoxia responses

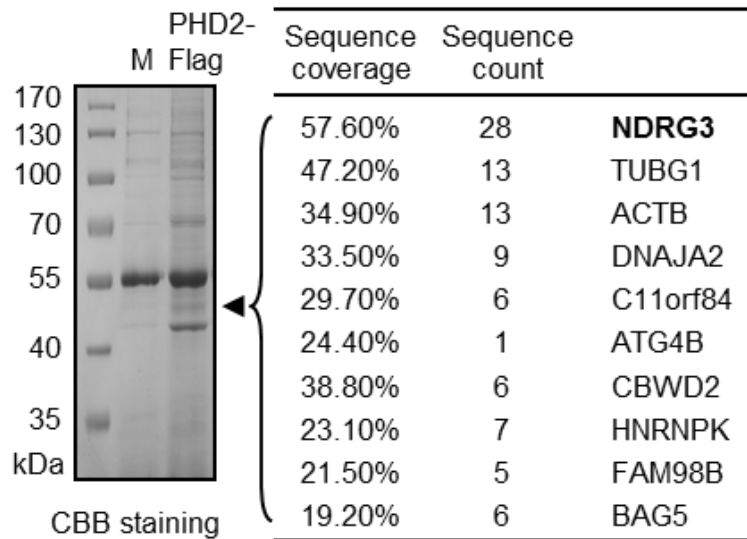


HIF independence of hypoxia responses

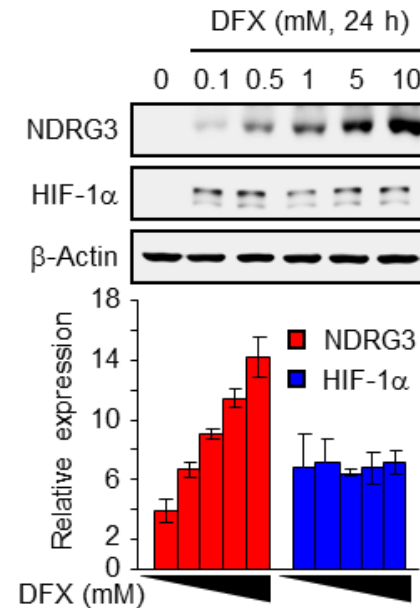
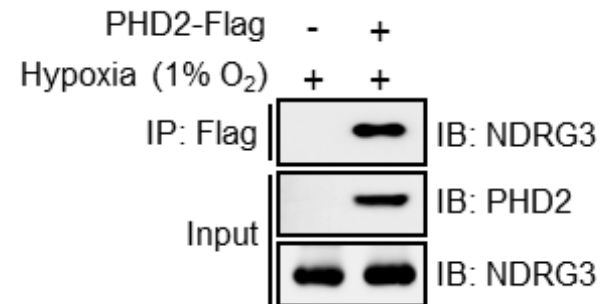
- Tumors derived from *HIF1A*^{-/-} ES cells had growth advantages owing to decreased hypoxia-induced apoptosis and increased stress-induced proliferation (Carmeliet et al., 1998).
- Angiogenesis was preserved in *HIF1A*^{-/-} ES cells (Hopfl et al., 2002).
- Induction of other pro-angiogenic factors such as IL-8 preserved the angiogenic response in HIF-1 α -deficient colon cancer cells (Mizukami et al., 2005).
- The pro-angiogenic factor, VEGF, can be induced via both HIF-dependent and -independent pathways (Mizukami et al., 2004).
- Multiple pathways and transcription factors (TFs) other than HIFs are known to respond to hypoxia to induce biological responses in a HIF-independent manner.
 - Oxygen-regulatable non-HIF TFs activated in hypoxia; NF- κ B, AP-1, CEBP (Cummins and Taylor, 2005)
 - Hypoxia-activated protein kinases; PKA, PKC, PI3K, Akt, JNK, Pyk2, Src, p38, ERK1/2 (Seta et al., 2002).

**Are there other oxygen-regulated pathways
that are, similar to HIF pathways, controlled
by PHD enzymes?**

Identification of NDRG3 as a PHD2-binding protein

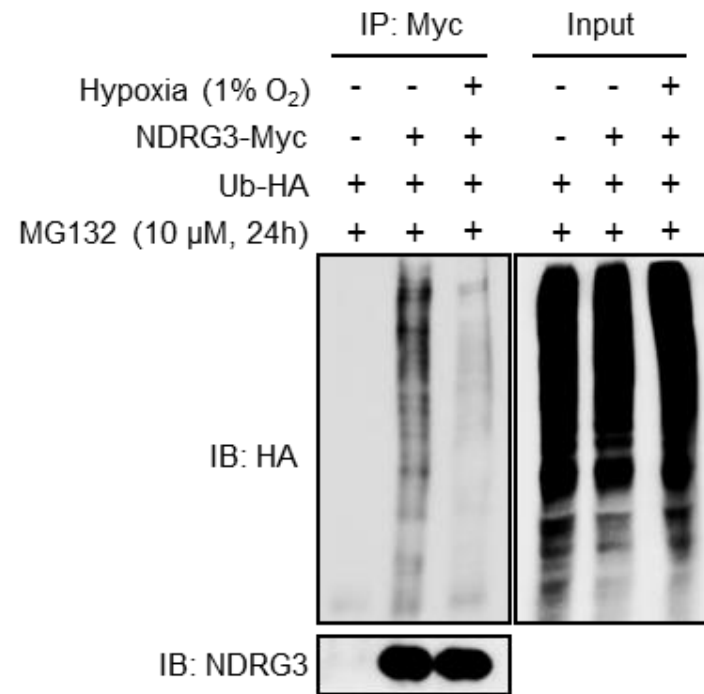
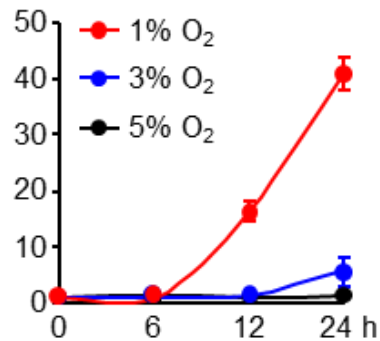
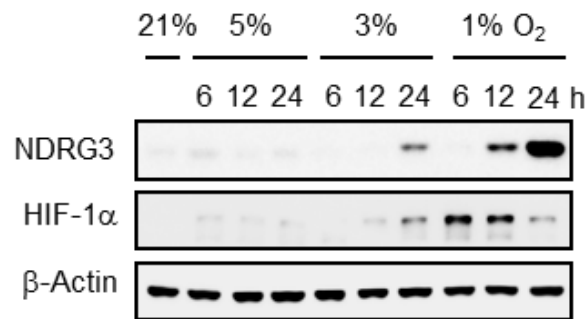


Immunoprecipitation – Mass spectrometry



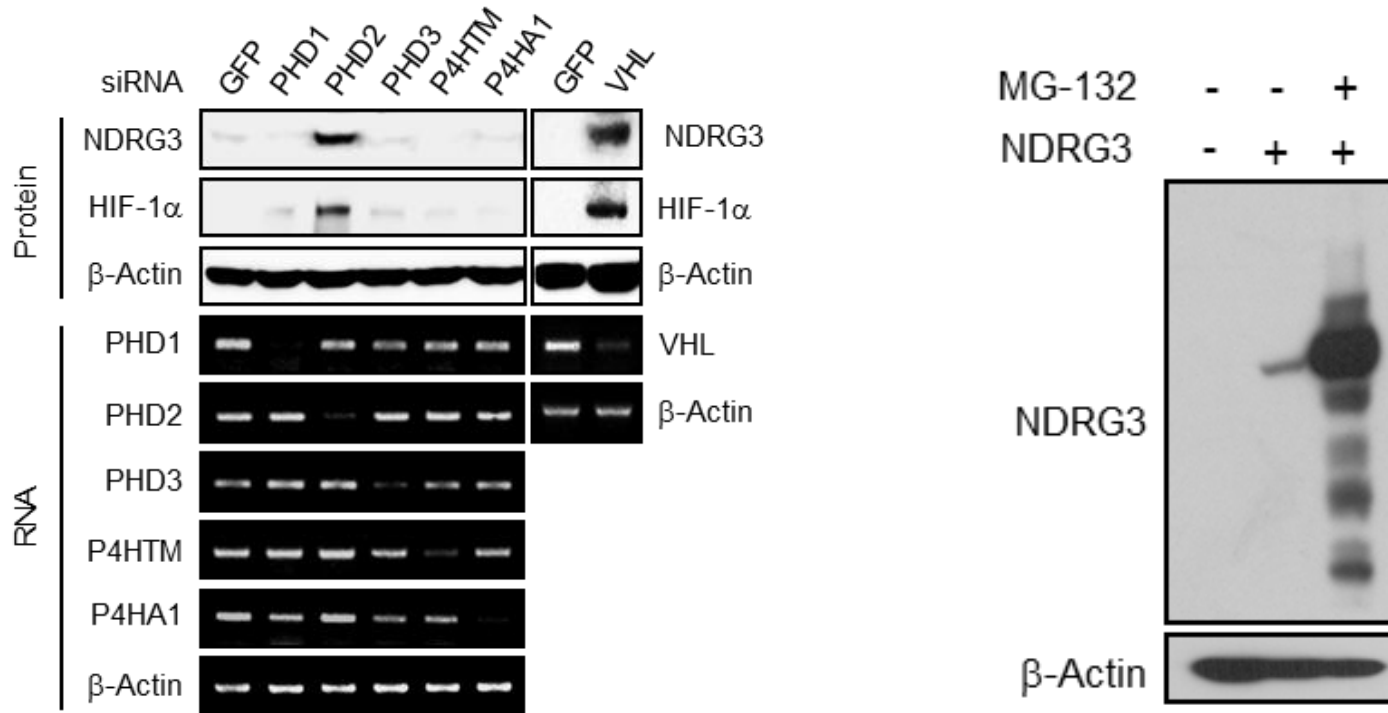
Regulation of NDRG3 expression in hypoxia

❖ Oxygen dependent expression

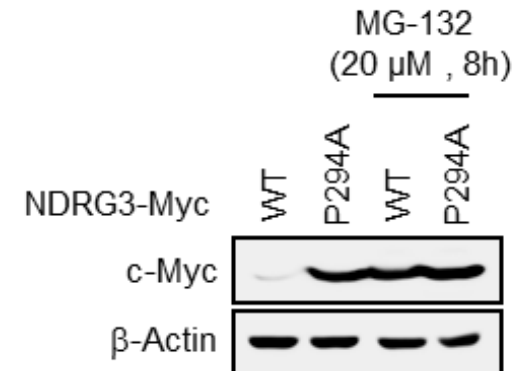
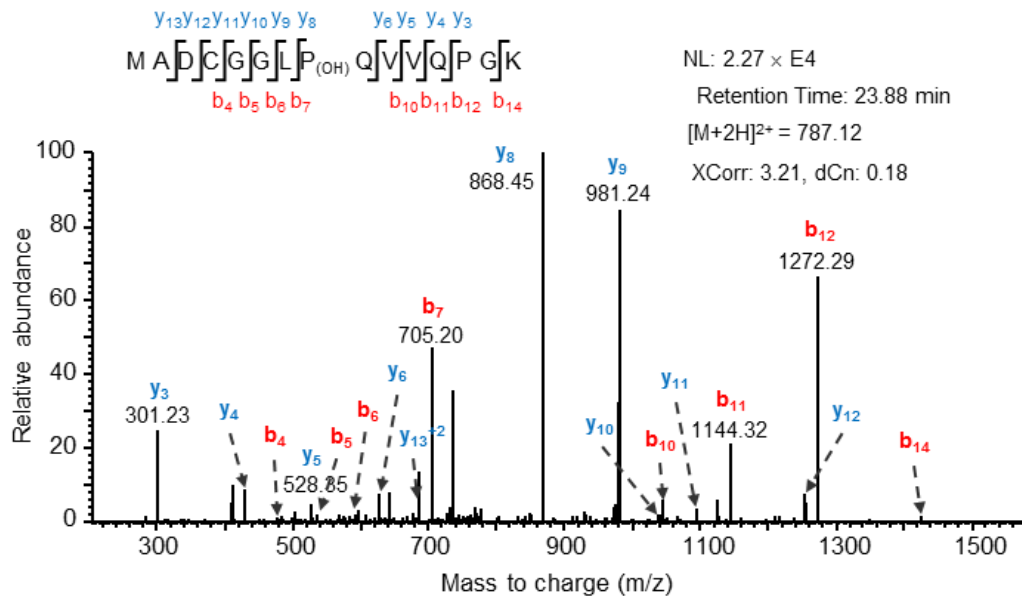


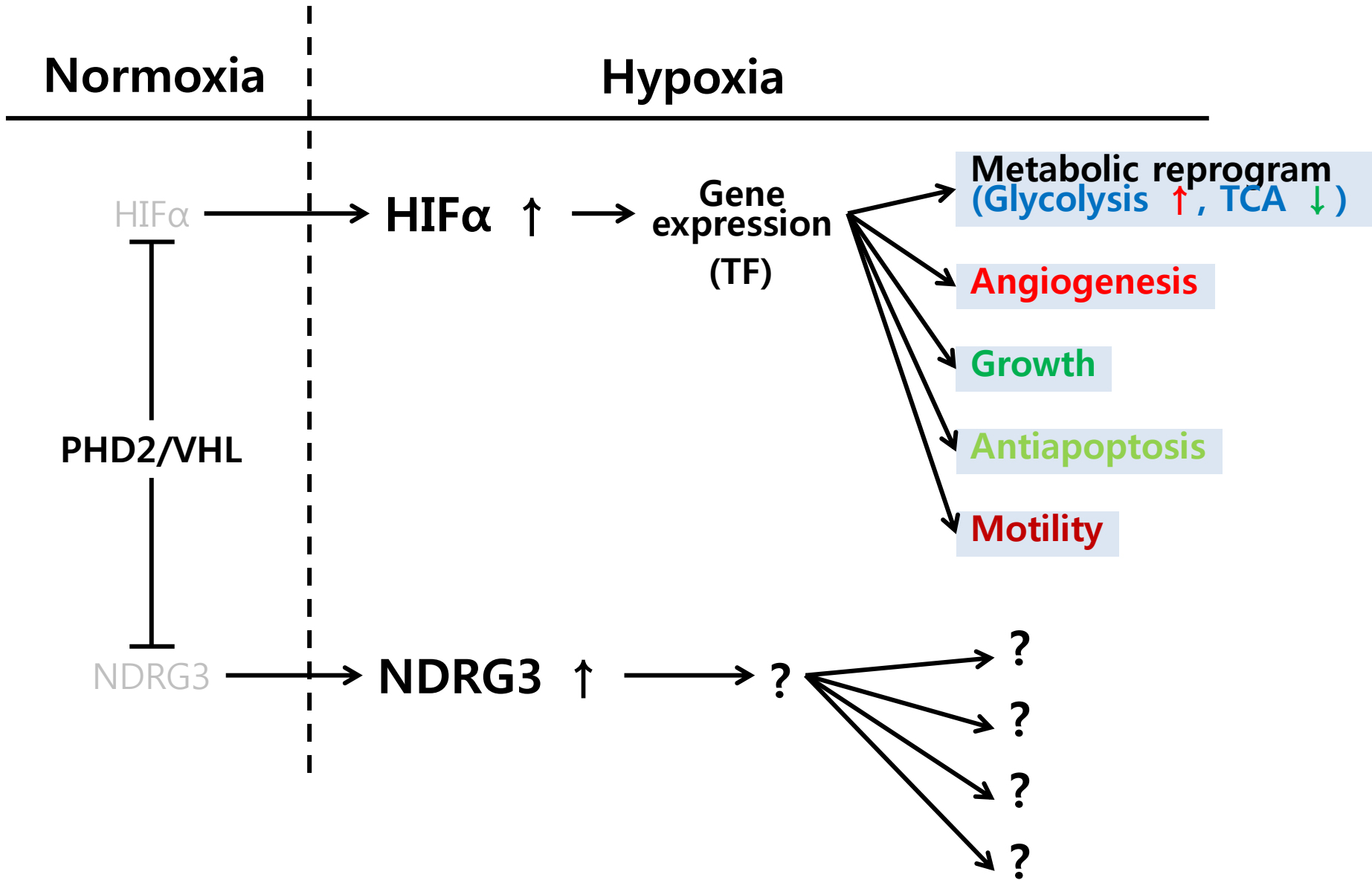
Regulation of NDRG3 expression in hypoxia

❖ Regulation by the PHD2/VHL-proteasome system



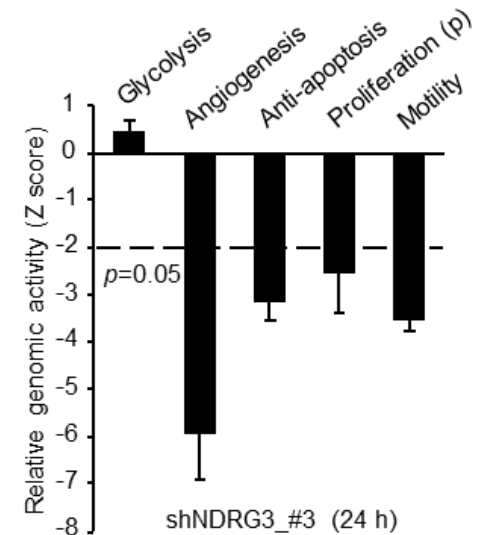
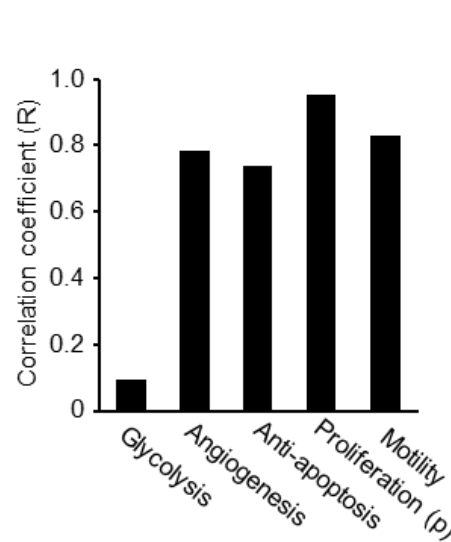
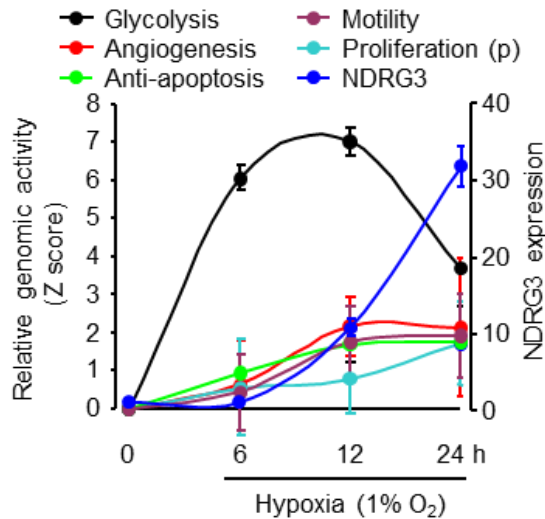
Regulation of NDRG3 expression in hypoxia



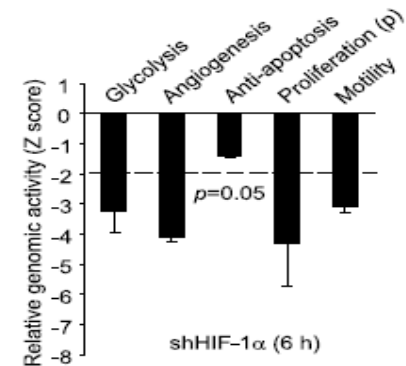


Role of NDRG3 in hypoxia response regulation

- ❖ Potential role of NDRG3 in angiogenesis, cell growth and survival under hypoxia but not in glycolysis

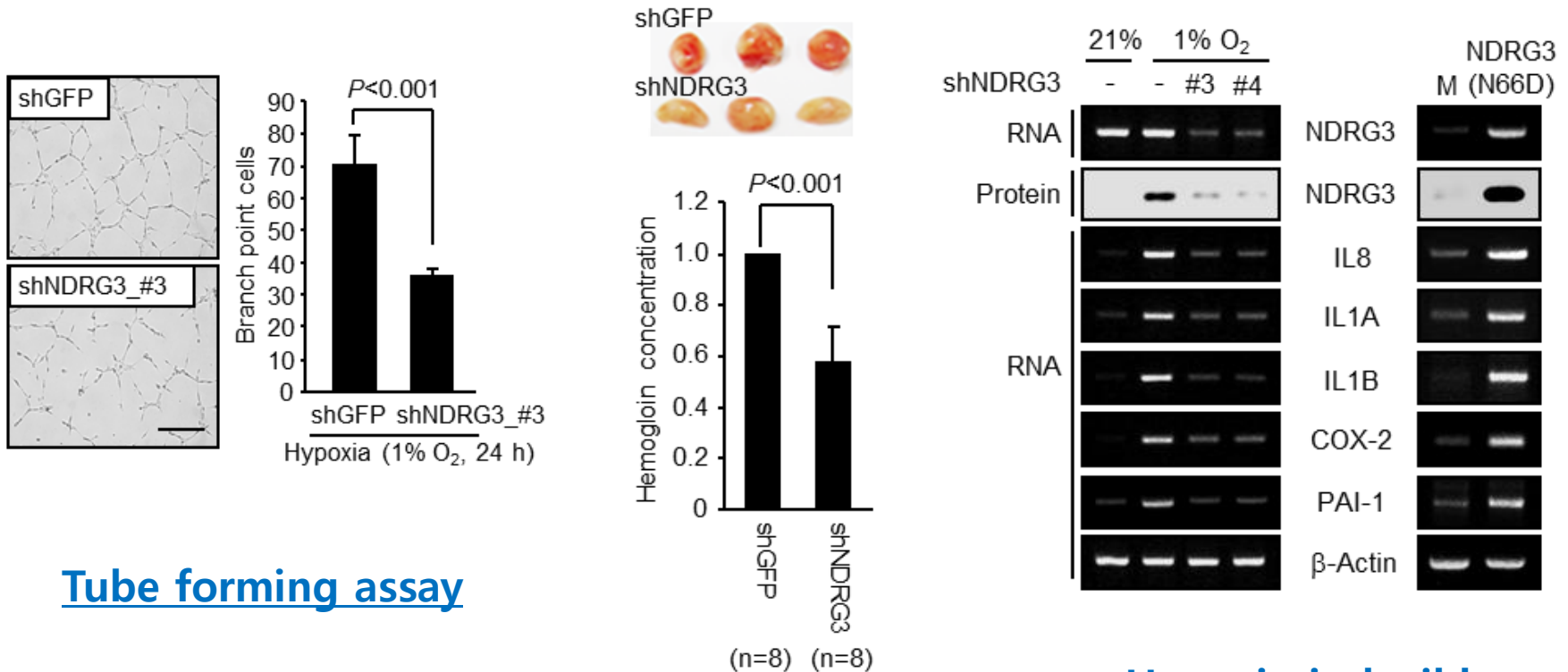


Gene set enrichment analysis
vs
NDRG3 protein expression



Role of NDRG3 in hypoxia response regulation

❖ NDRG3 activates hypoxic angiogenesis



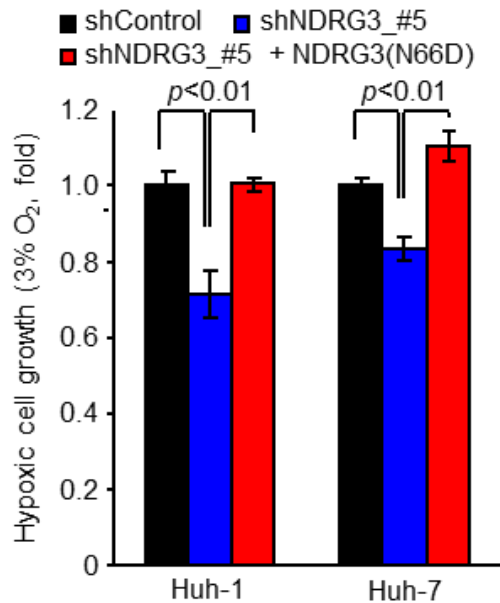
Tube forming assay

Matrigel plug assay

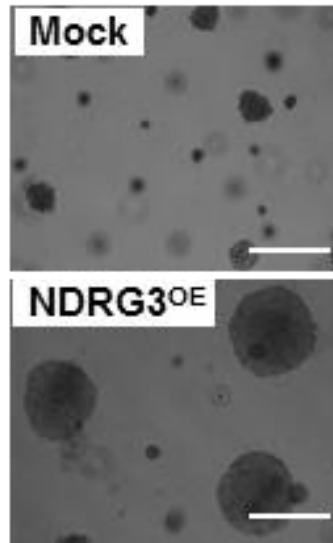
Hypoxia-inducible angiogenic gene expression

Role of NDRG3 in hypoxia response regulation

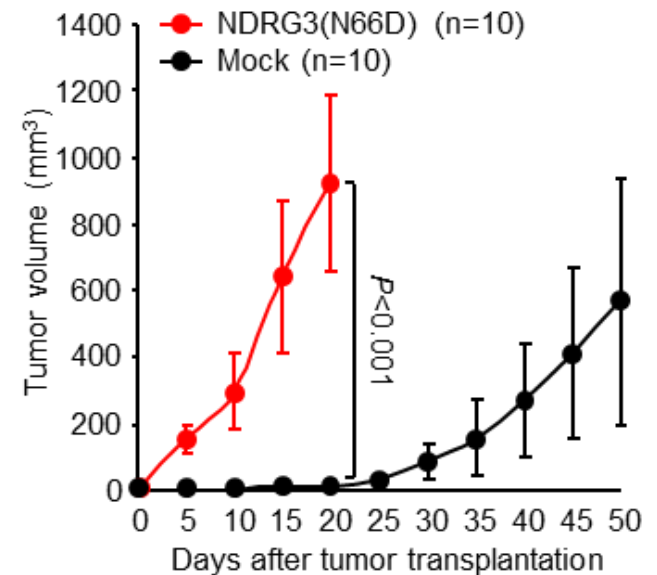
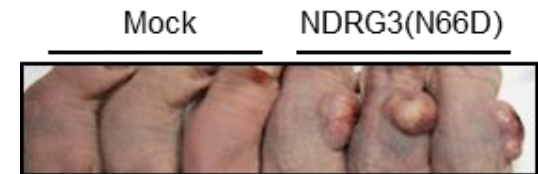
❖ NDRG3 promotes hypoxic cell growth



MTT assay

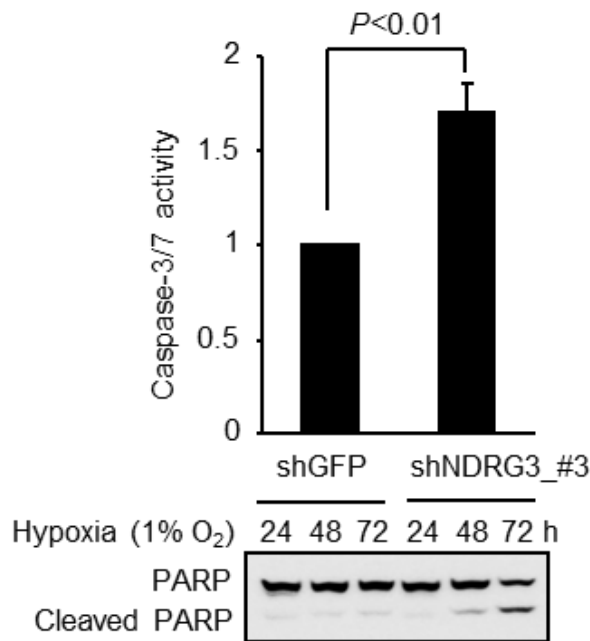


Colony forming assay

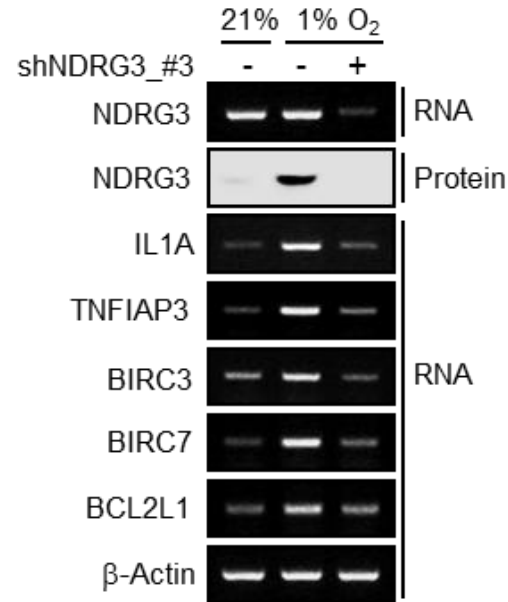


Role of NDRG3 in hypoxia response regulation

❖ NDRG3 mediates hypoxic cell survival

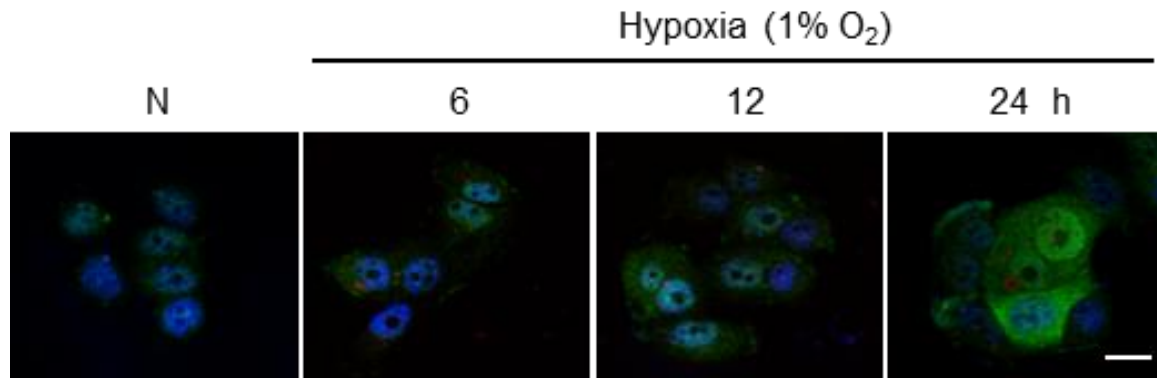
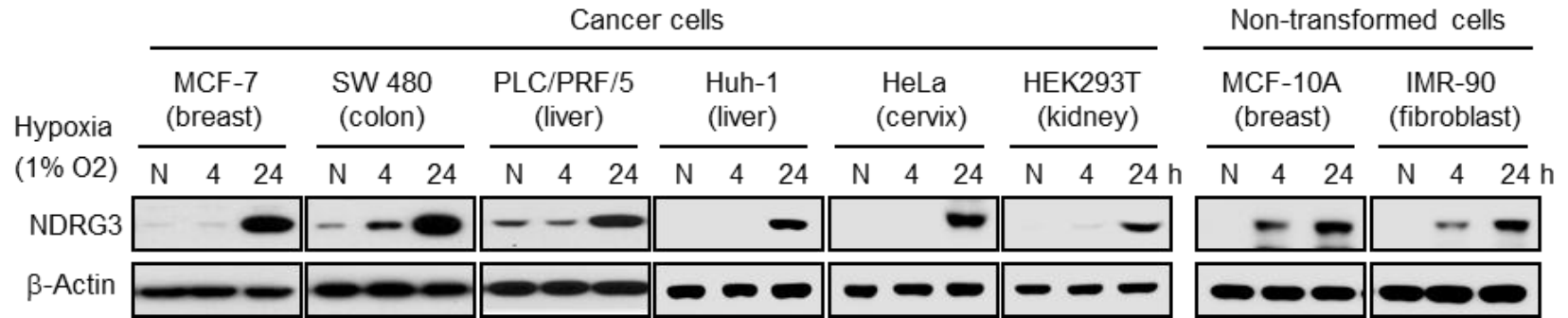


Apoptosis assays



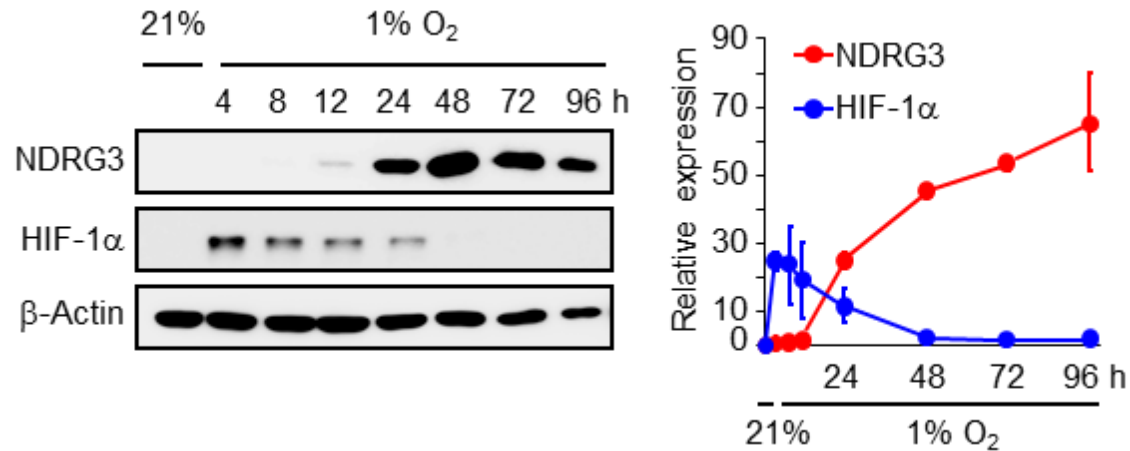
**Hypoxia-inducible
anti-apoptotic
gene expression**

Regulation of NDRG3 expression in hypoxia

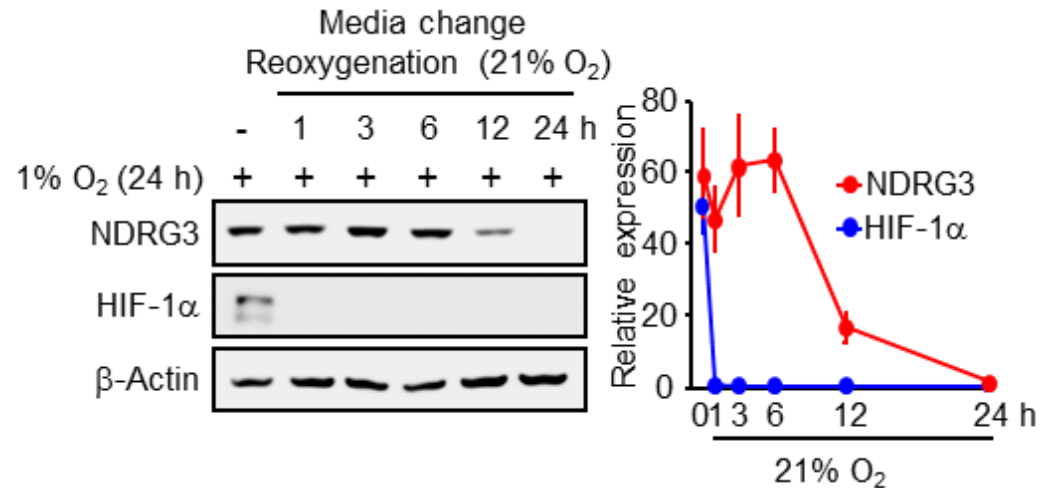


Lactate dependence of the NDRG3 protein dynamics under hypoxia

NDRG3 accumulation

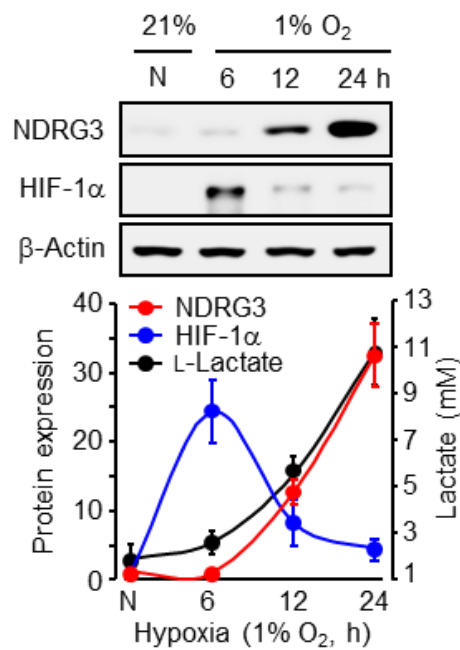


NDRG3 degradation

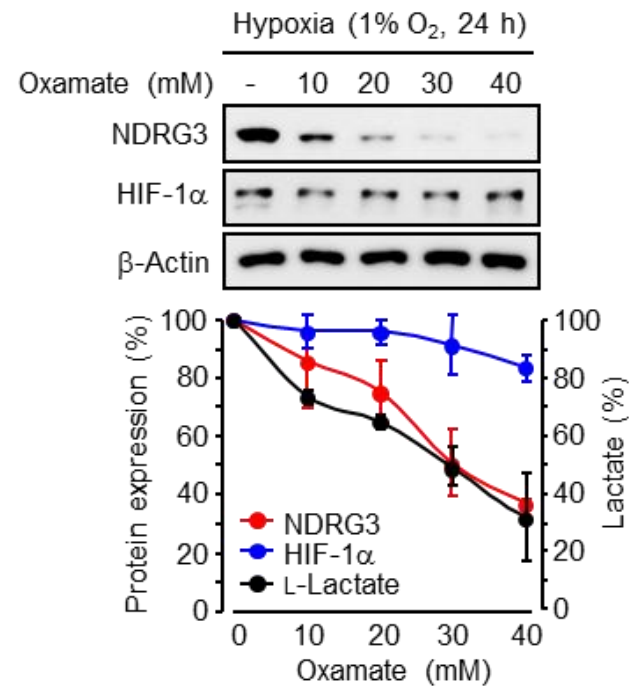


Lactate dependence of the NDRG3 protein dynamics under hypoxia

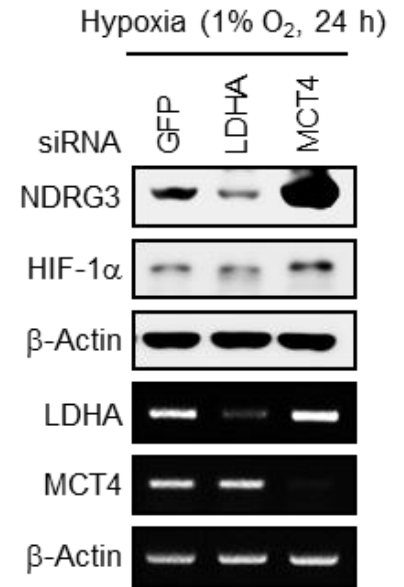
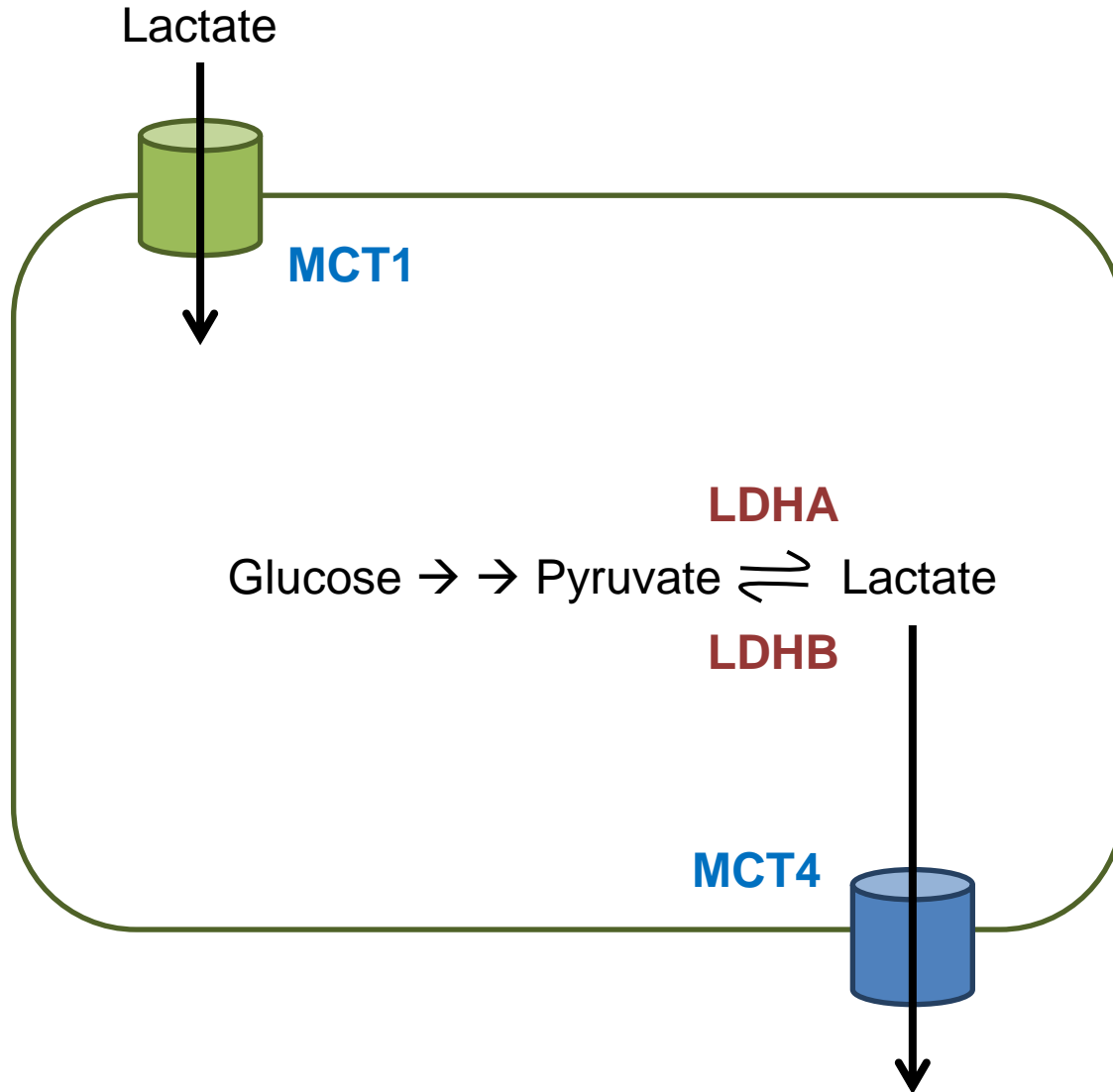
NDRG3 & Lactate accumulation



NDRG3 & Lactate suppression

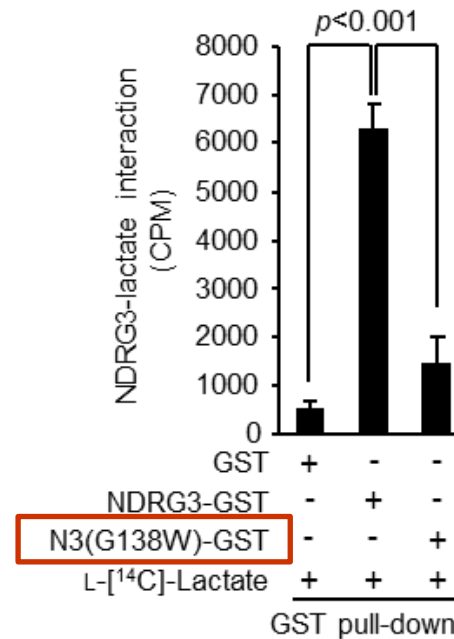
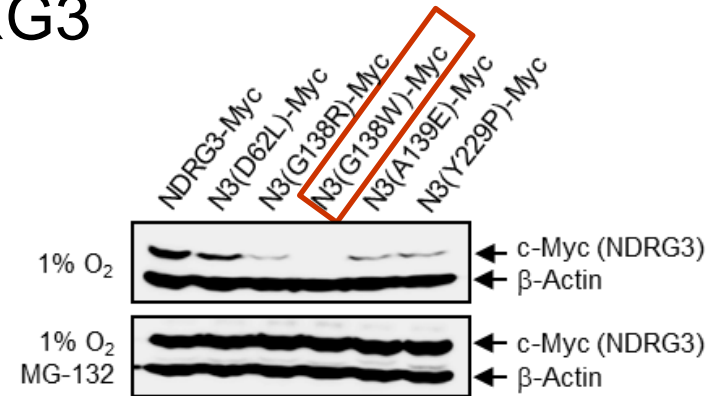
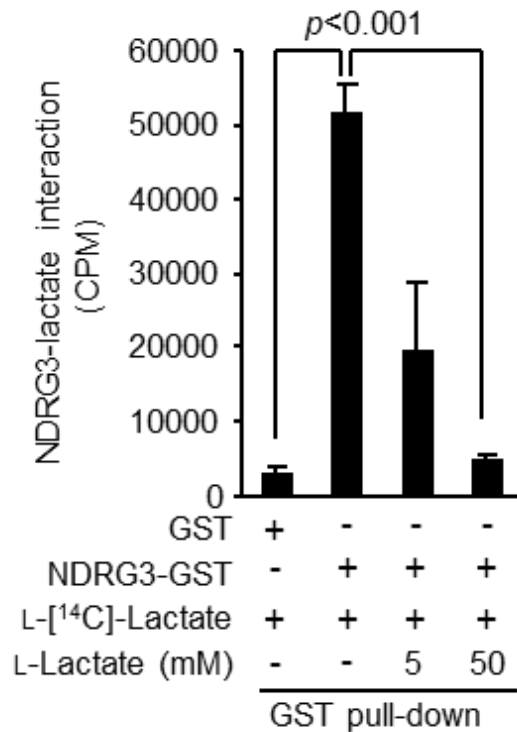


NDRG3 & Lactate metabolism



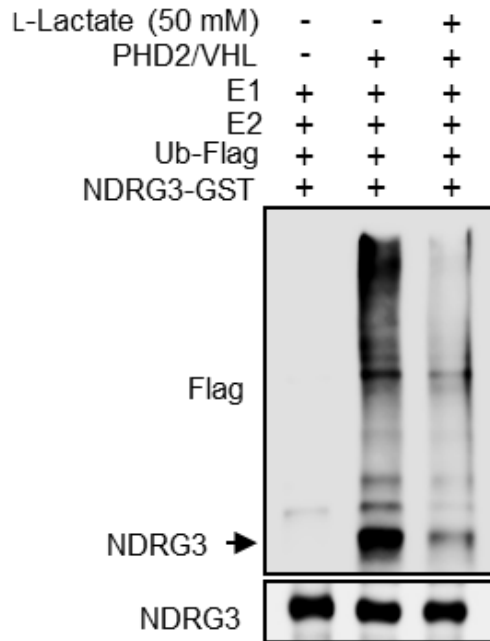
Lactate dependence of the NDRG3 protein dynamics under hypoxia

❖ Lactate directly binds to NDRG3

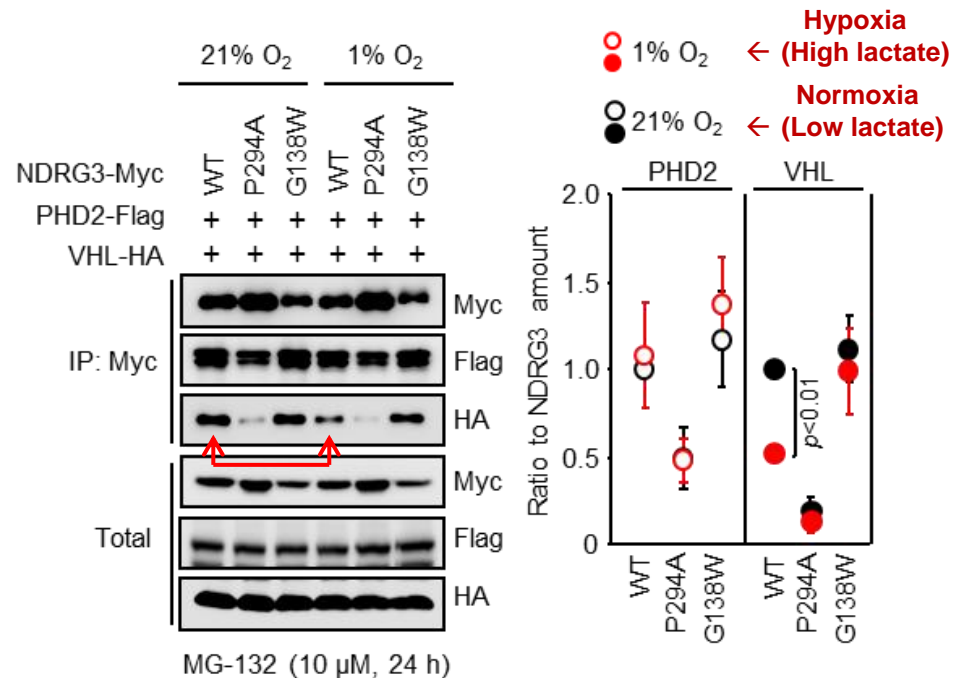


Lactate dependence of the NDRG3 protein dynamics under hypoxia

❖ Inhibition of PHD2/VHL-mediated NDRG3 modification by lactate in vitro

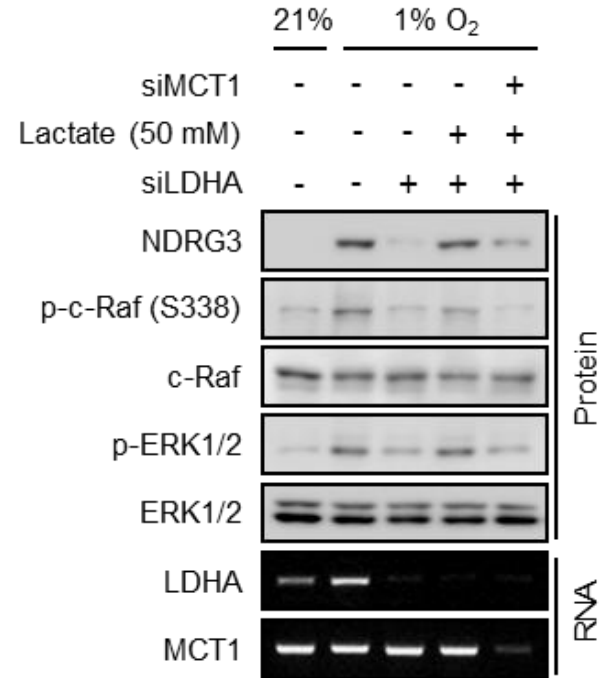
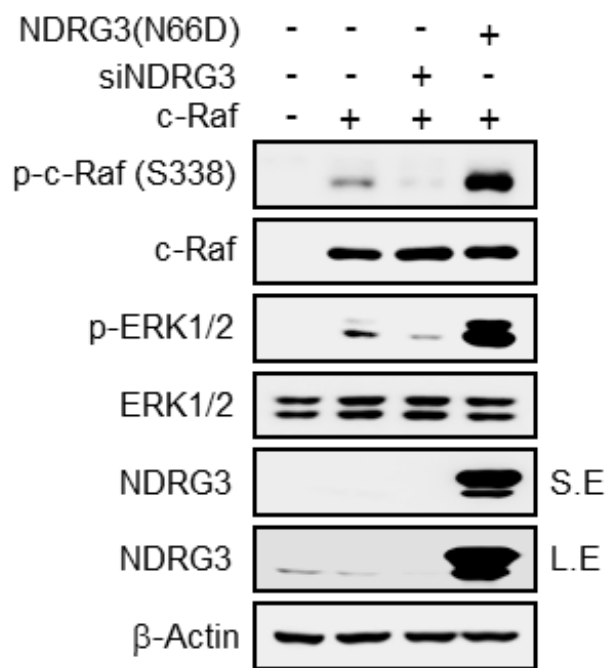


❖ NDRG3 interaction with PHD2 and VHL in Normoxia vs Hypoxia



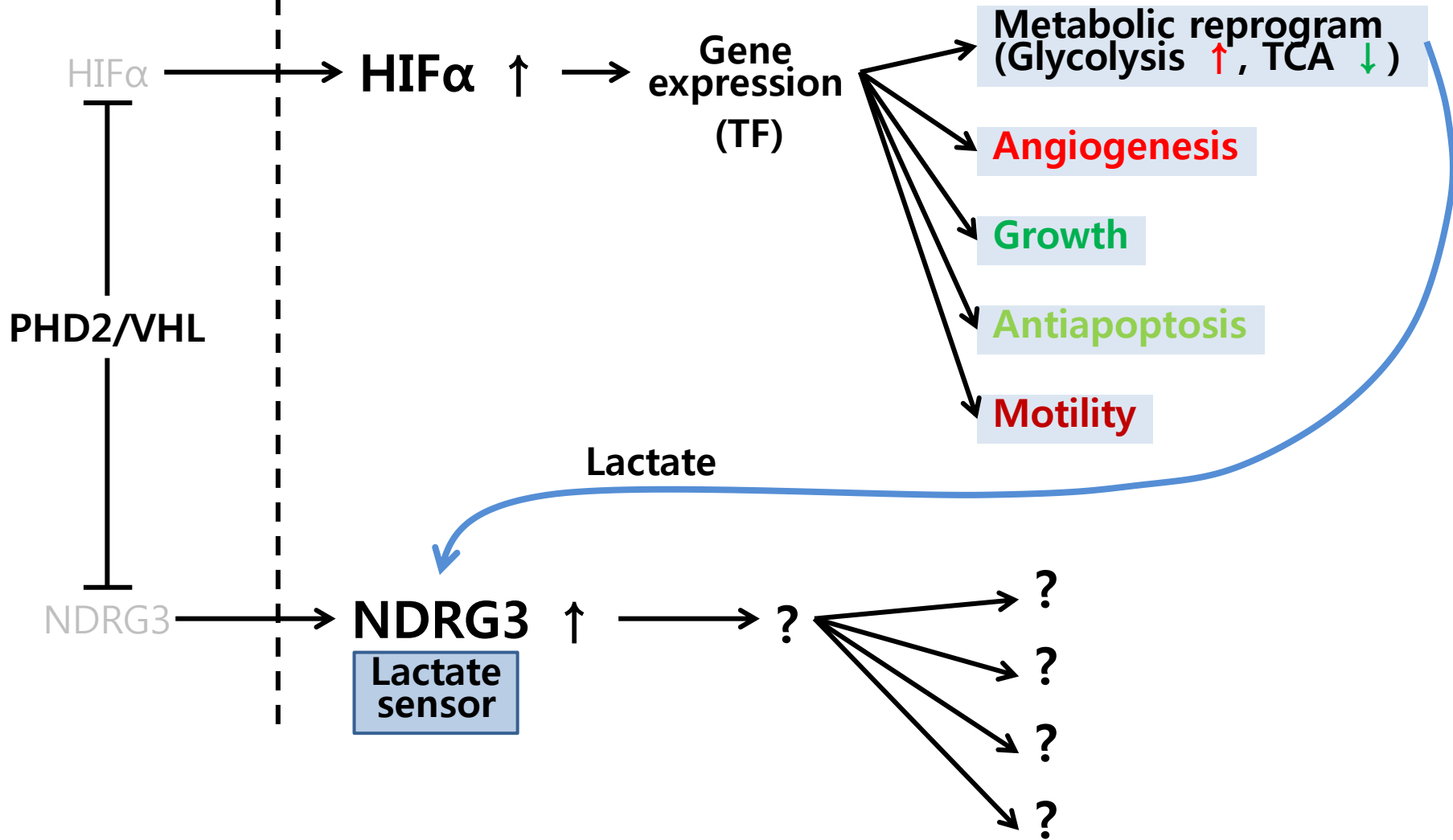
Cell signaling mediated by NDRG3

- ❖ NDRG3 activates Raf/ERK pathway during hypoxia in a lactate-dependent manner



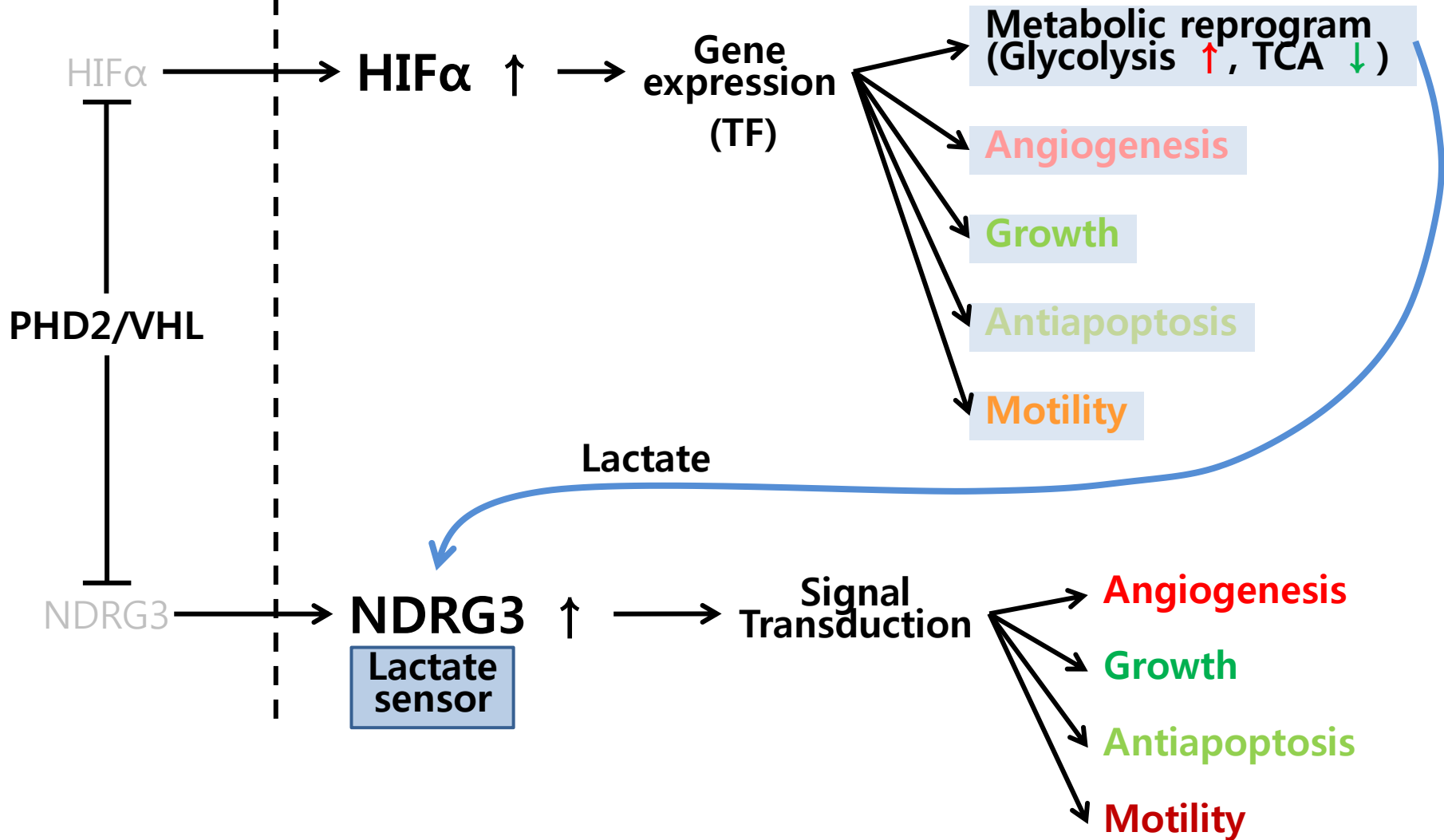
Normoxia

Hypoxia

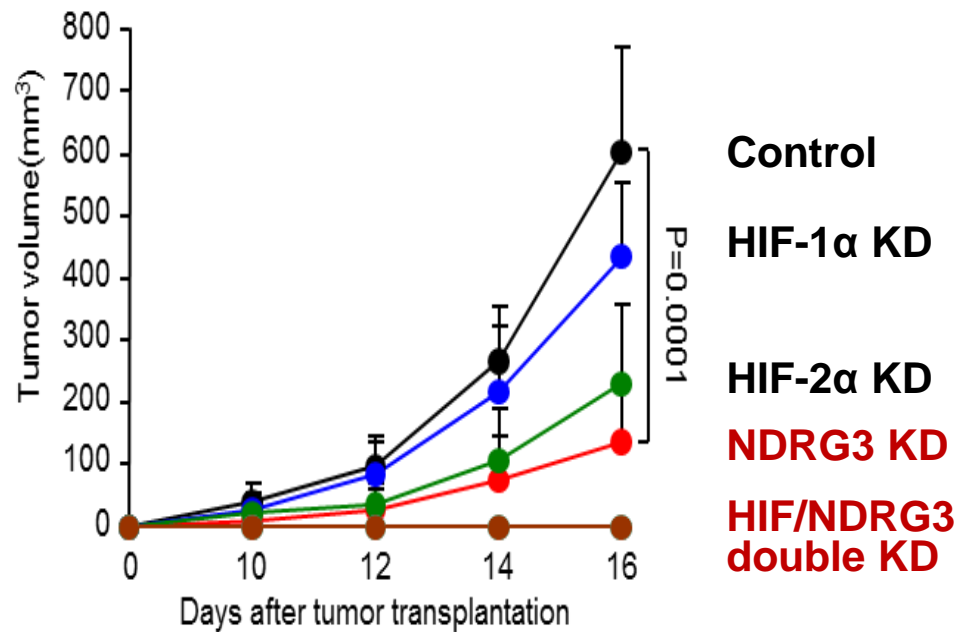


Normoxia

Hypoxia



Anticancer effects of inhibiting HIFs and/or NDRG3

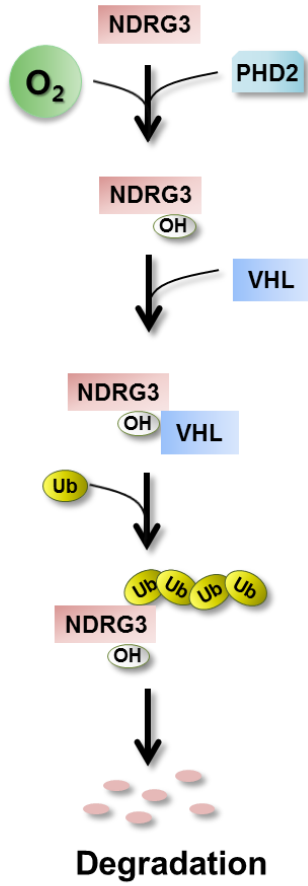


Lactate signaling system in hypoxia

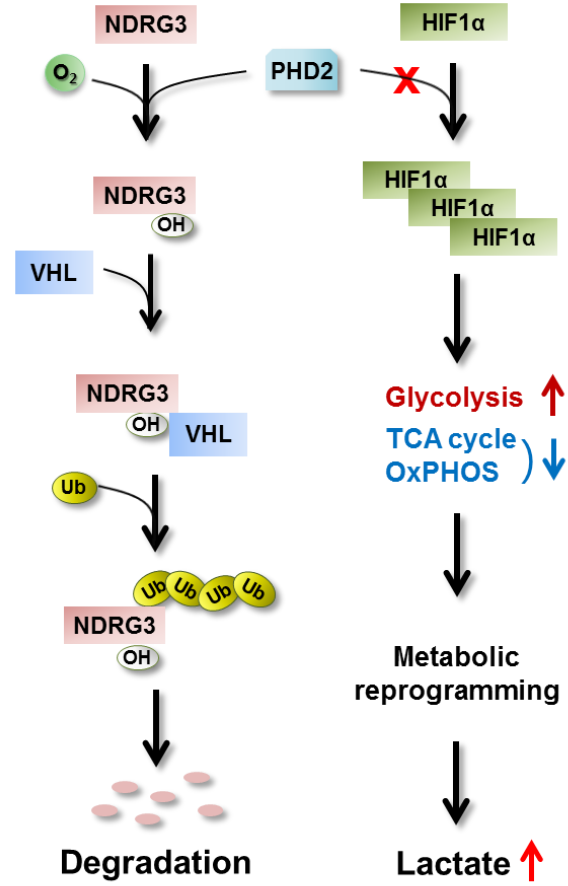
O₂

Lactate

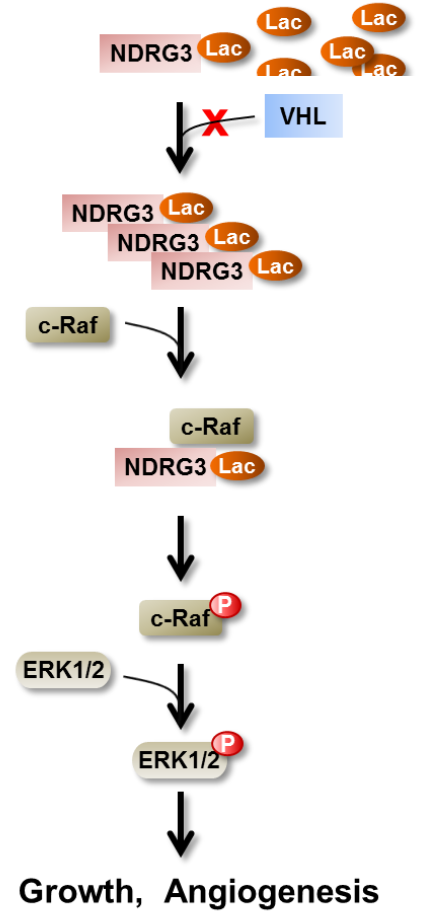
Normoxia



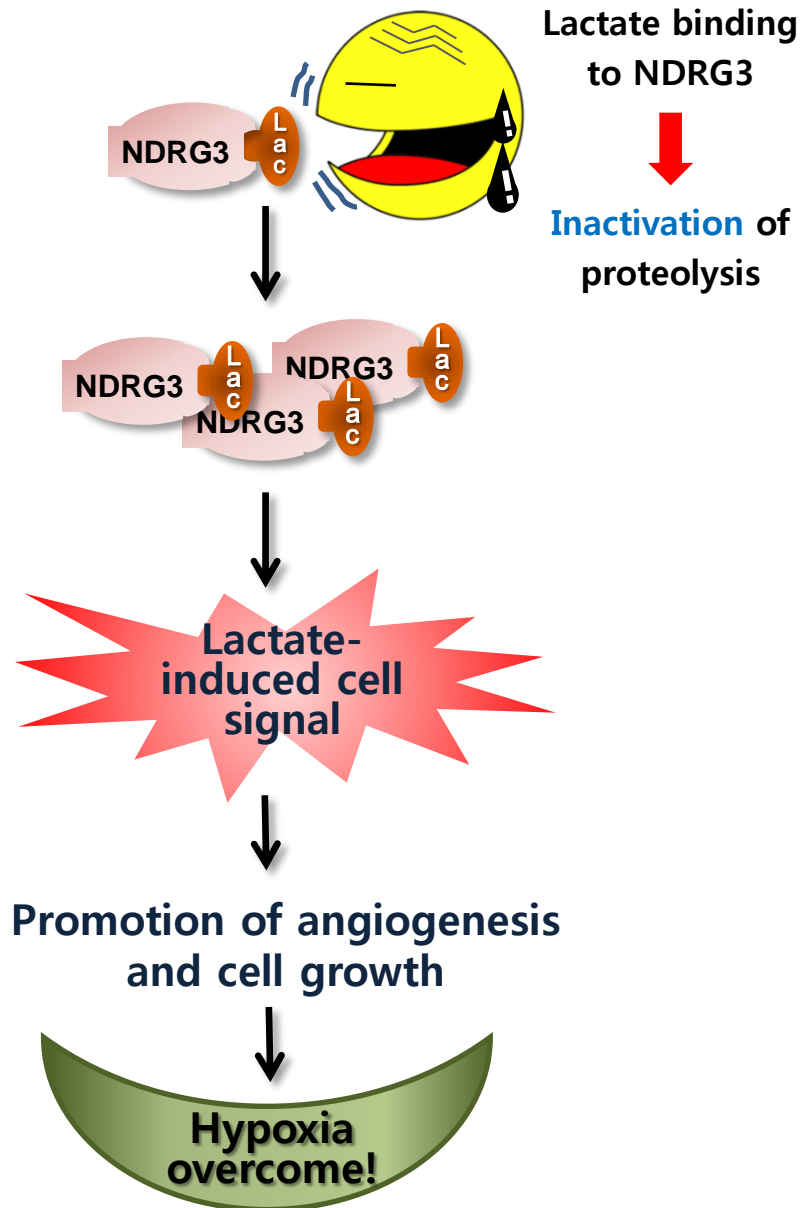
Early Hypoxia



Prolonged Hypoxia

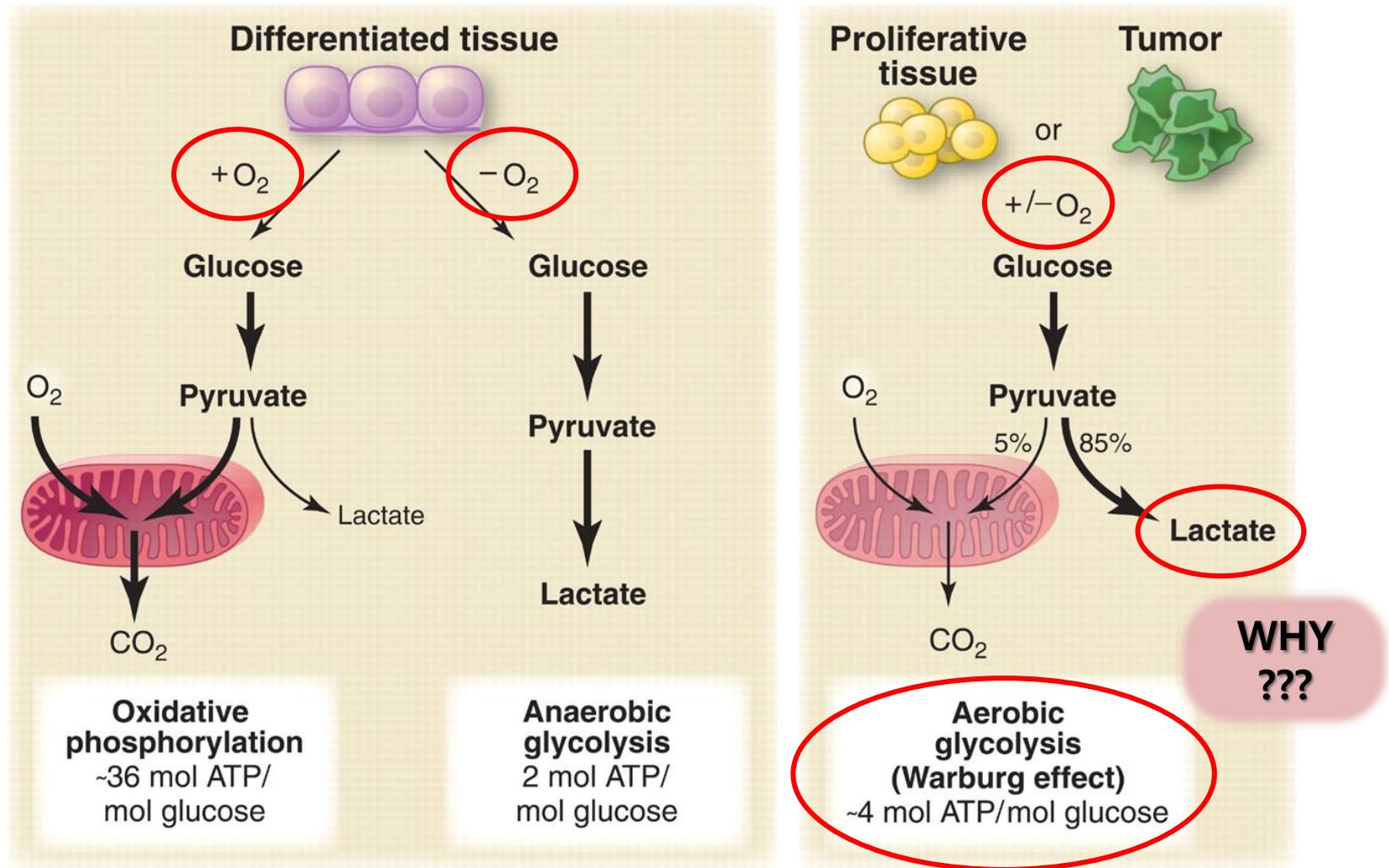


Hypoxia → Lactate accumulation

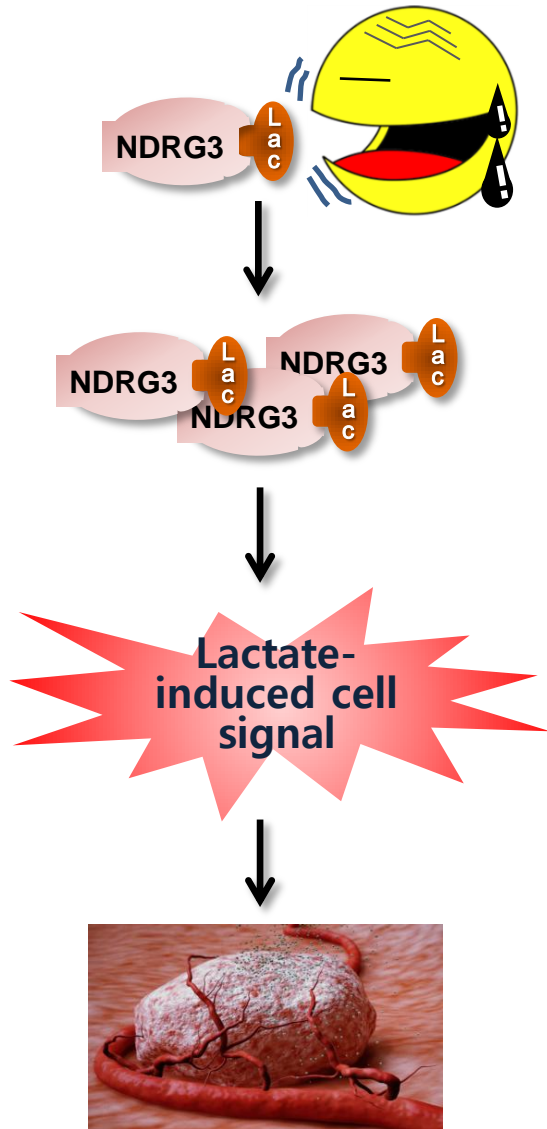


Altered metabolism in cancer cells

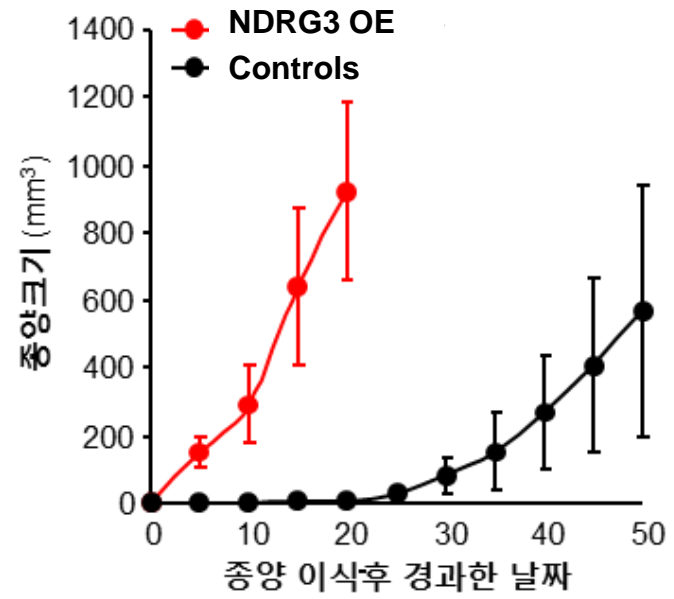
: Aerobic glycolysis (Warburg effect)



Solid tumors



Promotion of tumor angiogenesis and tumor growth



Lactate as a metabolic end product

- Produced in excess by muscle cells, red blood cells, brain, etc
 - AV Hill (Nobel Prize in Physiology or Medicine, 1922) :
Lactate build-up in muscle → Fatigue
- High lactate levels in blood
 - Hypoxia or abnormal blood pH
 - Pathological conditions : causing excess production or insufficient clearing of lactate from the blood
 - Sepsis, Shock, Heart attack, Heart failure, Kidney failure, Uncontrolled diabetes, Inherited metabolic or mitochondrial disorders



Novel functions of lactate

- **Role in tumor development**
 - As an alternative fuel
 - Activation of tumor angiogenesis
 - Activation of tumor metastasis
 - Immune suppression
- **Role in the regulation of brain functions**
 - Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell*. (2011)
 - Lactate-mediated glia-neuronal signalling in the mammalian brain. *Nat Commun*. (2014)
- **Role in muscle development**
 - Lactate regulates myogenesis in C2C12 myoblasts in vitro. *Stem Cell Res*. (2014)
- **Inhibition of lipolysis**
 - Lactate inhibits lipolysis in fat cells through activation of an orphan G-protein-coupled receptor, GPR81. *J Biol Chem*. (2009)

Cell signaling induced by lactate (Lactate-NDRG3-c-Raf-ERK axis)

✓ Potential biological significance

- Normal physiology

- Development; Pre- and post-natal
- Exercise physiology

- Pathology

- Cancers
- Inflammatory diseases
- Sarcopenia
- Ischemic diseases

KRIBB : **Dong Chul Lee**, Kyung Chan Park, Hyun Ahm Sohn,
Sangho Oh, Minho Kang, Suk-Jin Yang, Ye Jin Jang

GIST : Zee-Yong Park, Kyoung-min Lee

Inje Medical School : Yun Kyung Kang

Thank you...

**Ministry of Science, ICT and Future Planning of Korea,
National Research Foundation (NRF) :**

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Biomedical Technology Development Program

Senior Researcher Funding Program