HIF system AND IRON metabolism

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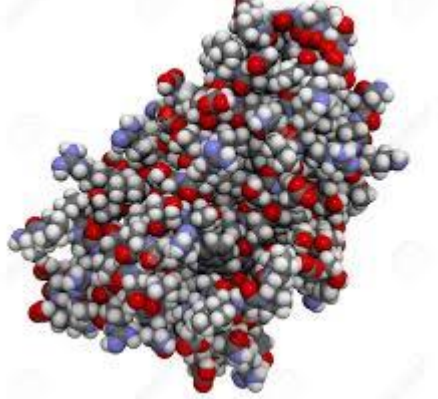
Anemia in CKD

➢The mechanisms of anemia in CKD are multifactorial.

- The progressive reduction of endogenous erythropoietin (EPO) levels play a preeminent role.
- an absolute iron deficiency due to blood losses or an impaired iron absorption
- an ineffective use of iron stores due to increased hepcidin levels
- a reduced bone marrow response to EPO due to uremic toxins
- a reduced red cell life span
- vitamin B12 or folic acid deficiencies

Hypoxia Inducible Factor System



- EPO is a glycoprotein (30.4 kDa) that binds to its receptor on the surface of erythroid progenitor cells mainly in the bone marrow, and serves as a key stimulus for red cell survival, proliferation and differentiation.

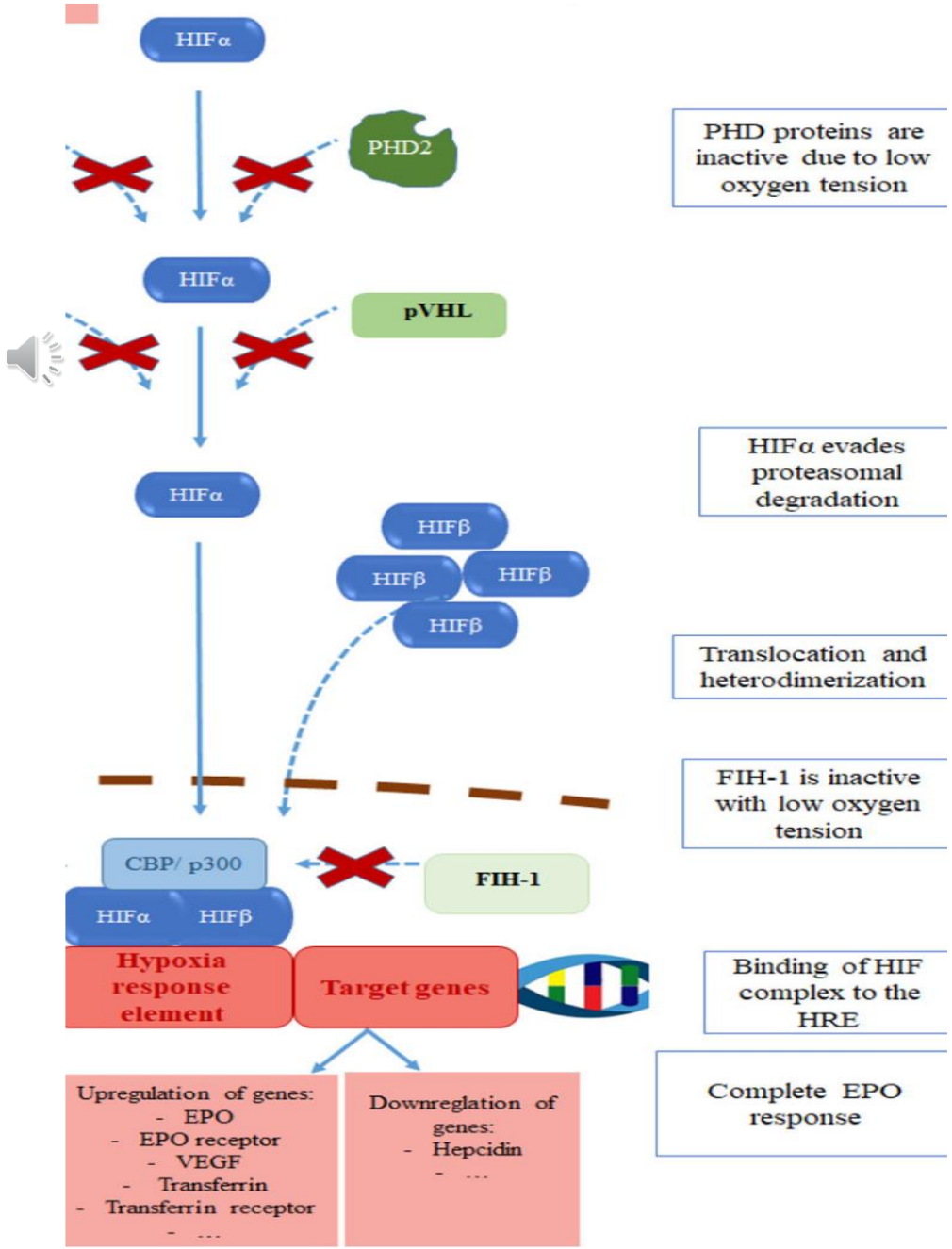
- EPO is produced by the fibroblast-like interstitial peritubular cells of the kidneys ,and by the perisinusoidal cells in the liver, in response to changes in tissue oxygen tension .

Hypoxia Inducible Factor System

- One of the most important factors that regulate its expression is the hypoxia-inducible factor (HIF) system, whose activity depends on the tissue oxygen levels.
- under hypoxia or anemic stress, the HIF1 binds to the EPO gene, and activates its expression.
- HIF1 is composed of two subunits:HIF1α and HIF1β.
- HIF1β is constitutively expressed whereas HIF1α is virtually absent under normoxic conditions.
- in low oxygen tension settings, HIF1α accumulates and translocates to the nucleus, where it binds to HIF1 β.

Hypoxia Inducible Factor System

- The HIF1 α-β heterodimer binds to DNA sequences called hypoxia response elements (HRE)
- regulating the expression of various hypoxia-sensitive genes, either downregulating or upregulating them.
- Among these hypoxia-sensitive genes is the EPO gene, which is activated, leading to an increased EPO production.
- Other genes that are transcriptionally upregulated by the HIF complex are those encoding EPO receptor, transferrin and transferrin receptor, vascular endothelial growth factor (VEGF) or endothelin-1


HIFα
PHD2
PHD proteins are inactive due to low oxygen tension
HIFα
pVHL
HIFα
HIFα evades proteasomal degradation
HIFβ
HIFβ
HIFβ
HIFβ
Translocation and heterodimerization
FIH-1 is inactive with low oxygen tension
CBP/ p300
FIH-1
HIFα
HIFβ
Hypoxia response element
Target genes
Binding of HIF complex to the HRE
Upregulation of genes:
- EPO
- EPO receptor
- VEGF
- Transferrin
- Transferrin receptor
- …
Downreglation of genes:
- Hepcidin
- …
Complete EPO response

Hypoxia Inducible Factor System

- Recent work has shown that the HIF transcription factors are key elements in the control of cell metabolism and function .

- An effect of HIF on total and LDL-cholesterol levels has also been described , probably in part by the effects of HIF on degradation of the rate-limiting enzyme, 3-hydroxy-3-methylglutaryl-CoA reductase

- similar to what has been observed in high altitude settings .

Hypoxia Inducible Factor System

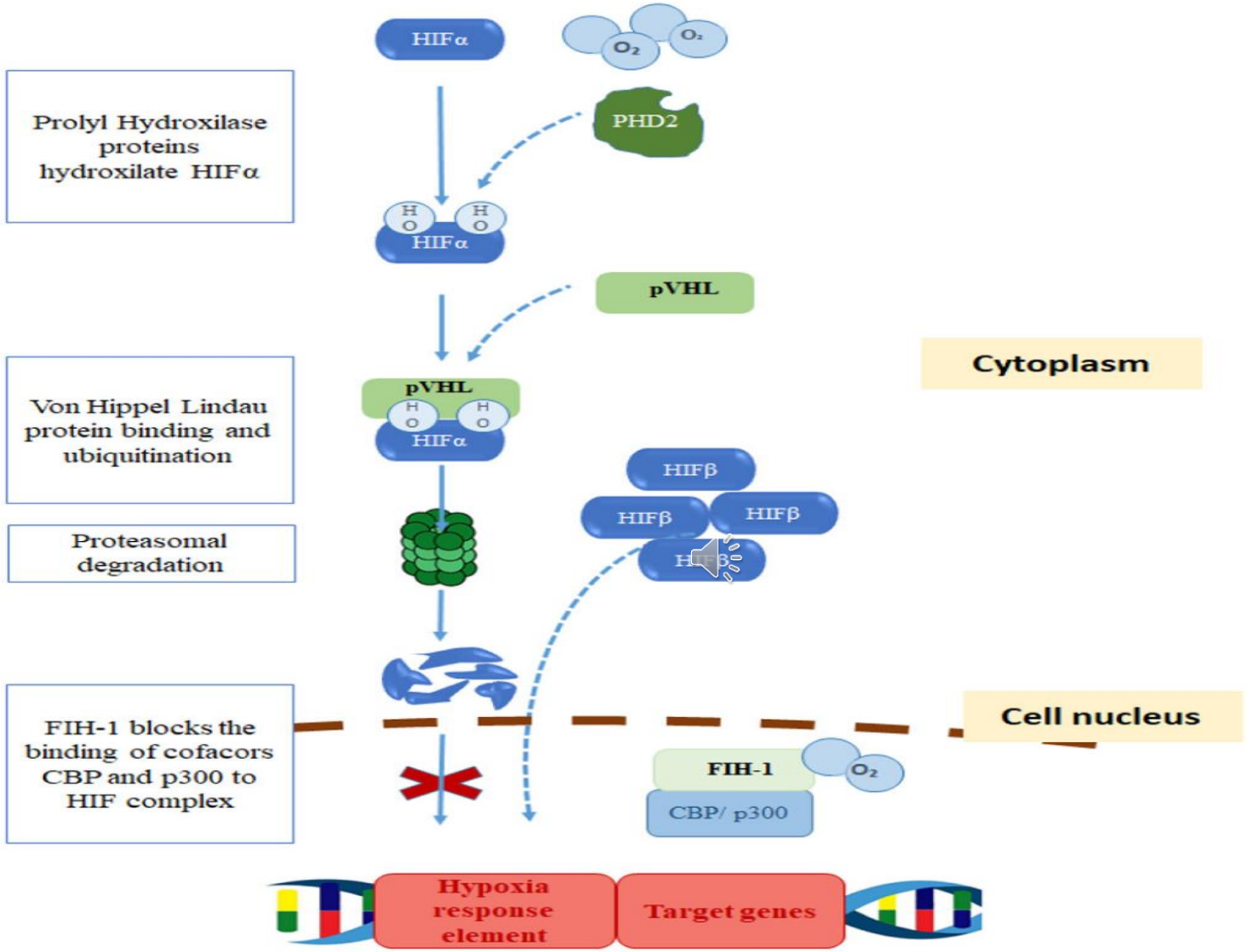
- **Under normoxic** conditions, HIF1α is degraded.
- HIF1α is hydroxylated at two proline residues.
- This hydroxylation is performed by specific HIF prolyl-hydroxylase enzymes called prolyl hydroxylase domain (PHD) enzymes that need the presence of oxygen, iron, and 2-oxoglutarate as cofactors.
- Three forms have been described: PHD1, PHD2, PHD3.
- **PHD2 is the main isoform regulating HIF activity** .

Hypoxia Inducible Factor System

- Once HIF1α is hydroxylated, the E3 ubiquitin ligase von HippelLindau (pVHL) binds HIF1α, and is targeted for proteasomal degradation.

- In contrast, under low oxygen tension the action of PHDs is prevented, allowing for HIF1α stabilization and translocation to the nucleus .

- This pathway is the target of the new so-called hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs)


Prolyl Hydroxilase proteins hydroxilate HIFα
Von Hippel Lindau protein binding and ubiquitination
Proteasomal degradation
FIH-1 blocks the binding of cofacors CBP and p300 to HIF complex
HIFα
O₂
PHD2
H O
HIFα
pVHL
Cytoplasm
HIFβ
Cell nucleus
FIH-1
CBP/ p300
Hypoxia response element
Target genes

Hypoxia Inducible Factor System

- HIF activity is also regulated through the hydroxylation at a carboxy-terminal asparagine residue of HIF1α by **factor inhibiting HIF (FIH) .**

- This hydroxylation of HIF1α occurs with normal oxygen levels and reduces its transcriptional activity.

- Indeed, it prevents HIF from recruiting transcriptional coactivators such as p300 or CBP, that are needed for the transactivation of hypoxia-responsive genes .
- **Angiotensin II**, which is often found to be increased in CKD patients, raises the production of reactive oxygen species, leading to an **inhibition of PHD enzymes**, and therefore, a rise in EPO levels

Hypoxia Inducible Factor System

- Three isoforms of HIFα have been described, that share similarities regarding oxygen-dependent hydroxylation: HIF1α, HIF2α, and HIF3α.

- All of them can bind to the HIFβ subunit.

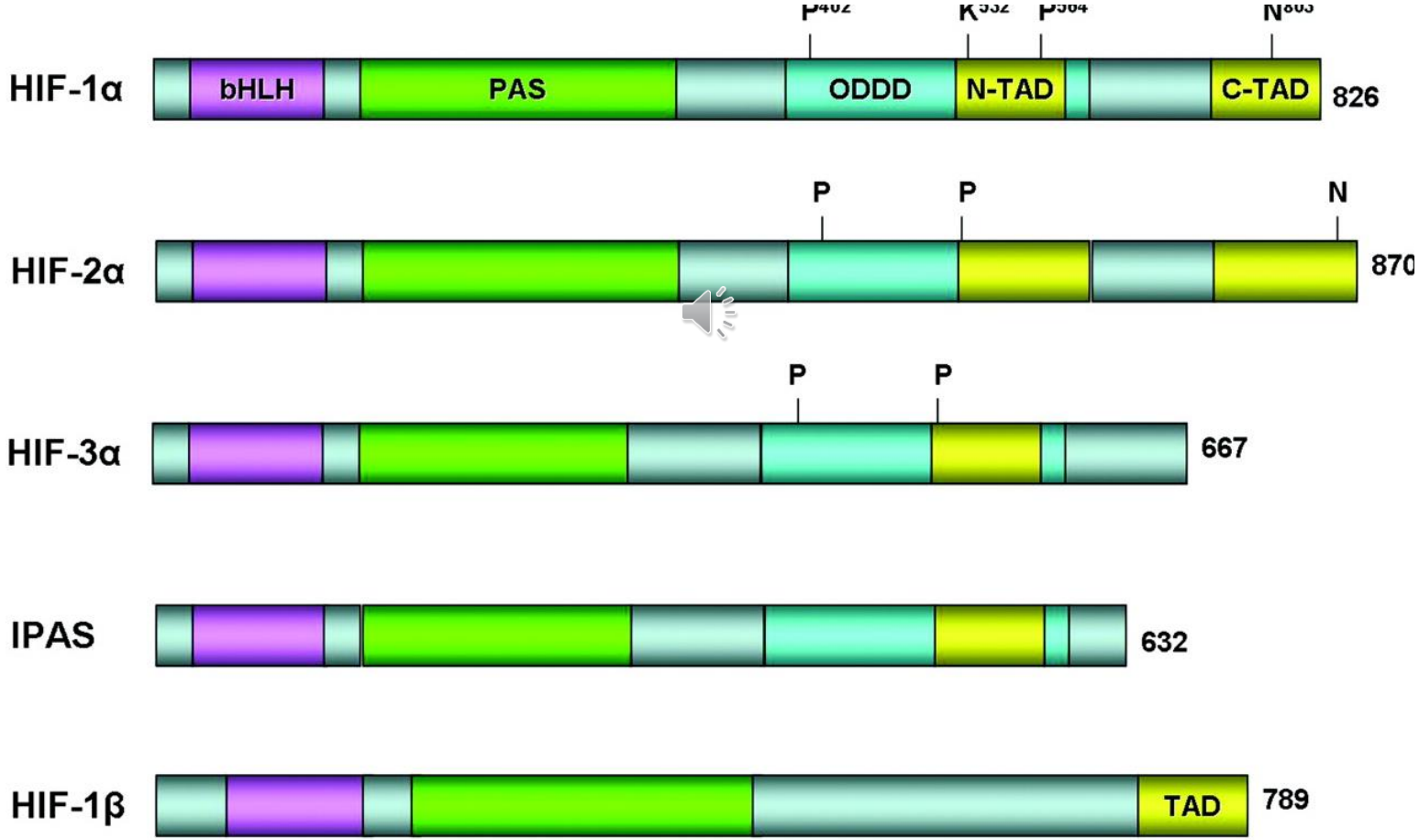
- there maybe some differences amongst them:
- while HIF1α and HIF2α activate gene transcription
- HIF3α downregulates HIF1α and HIF2α activity.
- their effect on the expression of some genes may also vary.

Hypoxia Inducible Factor System

- HIF2α may play a more important role than HIF1α in the regulation of EPO production, as it is specifically required for renal and hepatic production of EPO.

- The direct role of HIF3α on erythropoiesis has not been fully described.

- the expression of HIF2α and HIF3α is limited to several tissues, while HIF1α is ubiquitous


HIF-1α
bHLH
PAS
ODDD
N-TAD
C-TAD
826
P
P
N
HIF-2α
870
P
P
HIF-3α
667
IPAS
632
HIF-1β
TAD
789

EPO Production in CKD

- In CKD patients, EPO levels are inadequately low with respect

to the degree of anemia.

- EPO deficiency starts early in the course of CKD, but it appears that when eGFR falls below 30 ml/min per 1.73 m2 this deficiency becomes more severe .

- This absolute EPO deficiency can be caused by a decrease in the EPO production and/or by errors in EPO-sensing.

EPO Production in CKD

- CKD associates an alteration in oxygen delivery to the kidneys due to a reduced blood flow.

- This results in an adaptation of renal tissue to consume less oxygen and the subsequent maintenance of a normal tissue oxygen gradient.
- PHD enzymes remain active
- the HIF heterodimer is not formed
- the EPO gene is not activated.

EPO Production in CKD

- that hypoxia-induced EPO production is inhibited by some inflammatory cytokines : interleukin-la (IL-la), IL-l beta, transforming growth factor-beta (TGF-beta), and tumor necrosis factor-a (TNF-a).

- CKD itself leads to an increase of inflammation and immune activation molecules, which would inhibit hypoxia-induced EPO production.

- this mechanism of EPO production seems to be blunted rather than abolished in some CKD patients.

For instance, when exposed to high altitude or bleeding.

EPO RESISTANCE

- Some CKD patients may also present with a functional EPO deficiency or EPO resistance

- where normal range EPO levels coexist with low hemoglobin (Hb) levels , indicating that the bone marrow response to endogenous and exogenous EPO is blunted in patients with CKD.

EPO RESISTANCE

➢The mechanisms that have been hypothesized for the EPO resistance are various:

- **Proinflammatory cytokines** induce apoptosis as well as a to have a direct toxic effect via the induction of labile free radical nitric oxide on erythroid progenitor cells
- **Proinflammatory cytokines** downregulate the expression of EPO receptor on their surface too.
- **cytokines** can induce the production of antagonistic peptides that bind to the EPO receptor, and inhibit the EPO-dependent proliferation .
- **hepcidin** contribute to EPO resistance, by directly inhibiting erythroid progenitor proliferation and survival .
- **neocytolysis** is a homeostatic physiological process that leads to selective hemolysis of young circulating red blood cells, that has been found to contribute to resistance in CKD patients receiving exogenous EPO

Iron Metabolism

- Iron is required for an adequate erythropoietic response to EPO,

and in anemic conditions having iron deficiency corrected allows

lower exogenous EPO supplies .

- iron is required in other essential non erythropoietic

effects that may help explain symptoms such as impaired exercise performance, cognitive impairment or decreased quality of life, and an increased risk in hospitalization or death in patients with heart failure and reduced ejection fraction, independent of anemia.

Iron Metabolism

- Iron is an essential component of myoglobin, which transports oxygen in the muscle cells.
- Iron plays a substantive role in several oxidative reactions affecting intracellular metabolism, such as the electron transport chain or oxidative phosphorylation.
- Iron is involved in different mechanisms of DNA synthesis, degradation and repair.
- iron is an important component of cytochrome P450 family .
- ***there is an increasing evidence from observational studies that iron deficiency is associated with worse outcomes in CKD patients .***

Iron Metabolism

- Most of the iron requirements are provided by recycling the

iron present in **senescent erythrocytes** and the release of iron

from storage sites

- The proportion of iron that comes from the dietary uptake is much smaller.

- there is no physiological mechanism to regulate iron excretion.
- It is lost from **the desquamation** of **intestinal epithelial cells**, **skin** cells and blood loses and dietary iron absorption, which is regulated by hepcidin, compensate these loses.

Iron Metabolism

- The iron content of macrophages from the phagocytosis of senescent red blood cells, hepatocytes or enterocytes (dietary iron absorbed in the duodenum) is released into the circulation by **ferroportin**, the **only iron exporter known.**

- Iron is then transported through the circulation by **transferrin**, and delivered into target cells by transferrin binding to **transferrin receptor**.

- Transferrin receptors are regulated by intracellular iron quantity and cell growth.

HEPCIDIN

- A circulating acute-phase protein produced in the liver**, hepcidin**, is the key regulator of iron metabolism.

- Hepcidin is thought to decrease the absorption of iron in the doudenum by **downregulating** the expression of apical divalent metal transporter 1 **(DMT1)** in the enterocyte.

- Besides absorption, hepcidin also plays a role in iron storage.

- hepcidin promotes the internalization of **ferroportin i**nto the cell for its degradation, and preventing iron form exiting into the circulation from enterocytes, macrophages or other iron stores.

- Iron overload increases hepcidin levels, whereas iron deficiency reduces its concentrations .

IRON DEFICIENCY

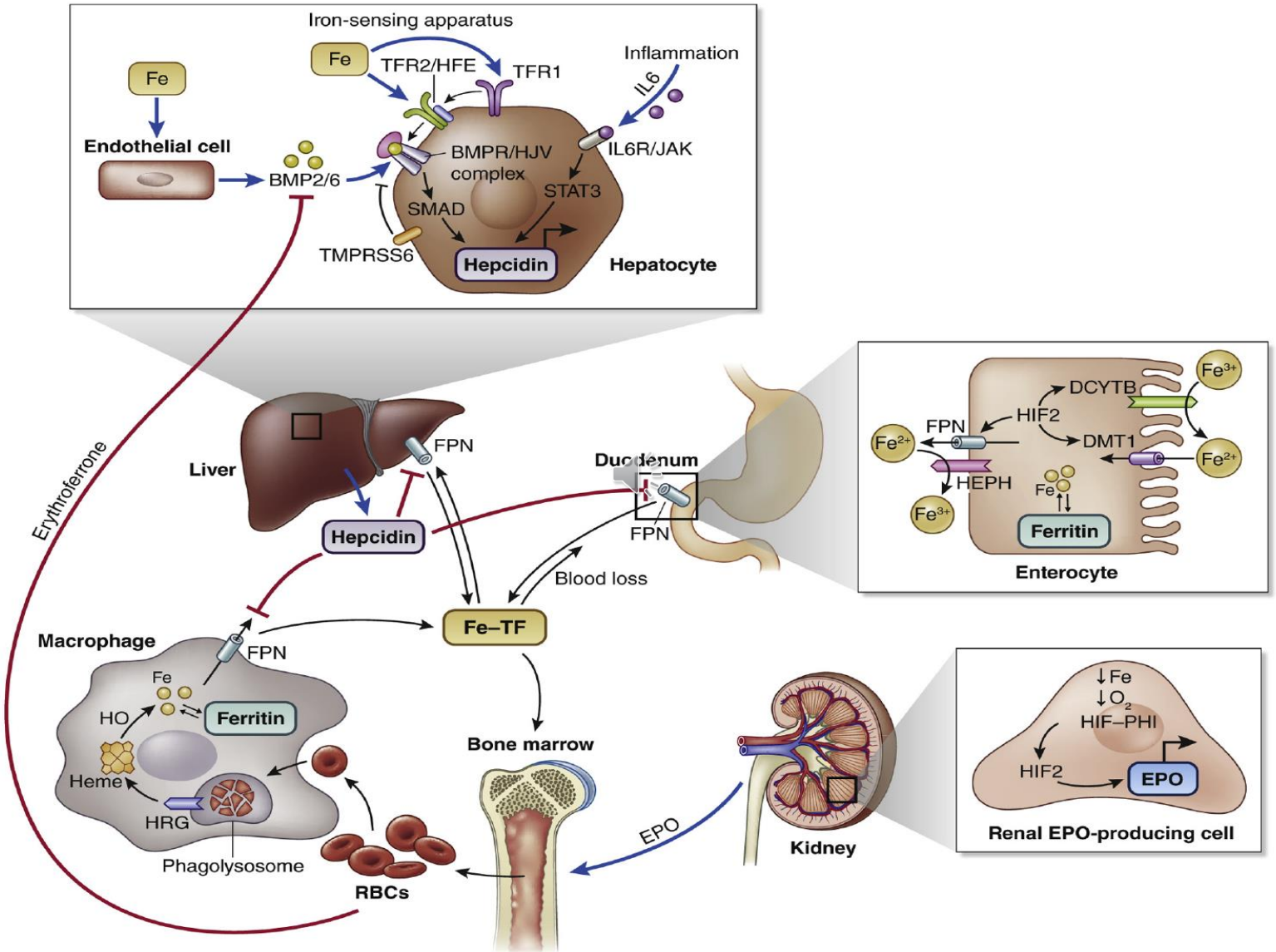
- Absolute and relative iron deficiency are frequent conditions in CKD patients.

- Proinflammatory cytokines contribute to a functional iron deficiency in several ways:

- They stimulate the hepatic synthesis of hepcidin,
- they induce the expression of DMT1 in macrophages,
- and induce the expression of ferritin,
- and inhibit that of ferroportin.
- promote the uptake of iron bound to transferrin into macrophages, via the transferrin receptor .
- hepcidin is eliminated by kidney and its clearance is reduced as eGFR declines.

ERYTHROFERRON

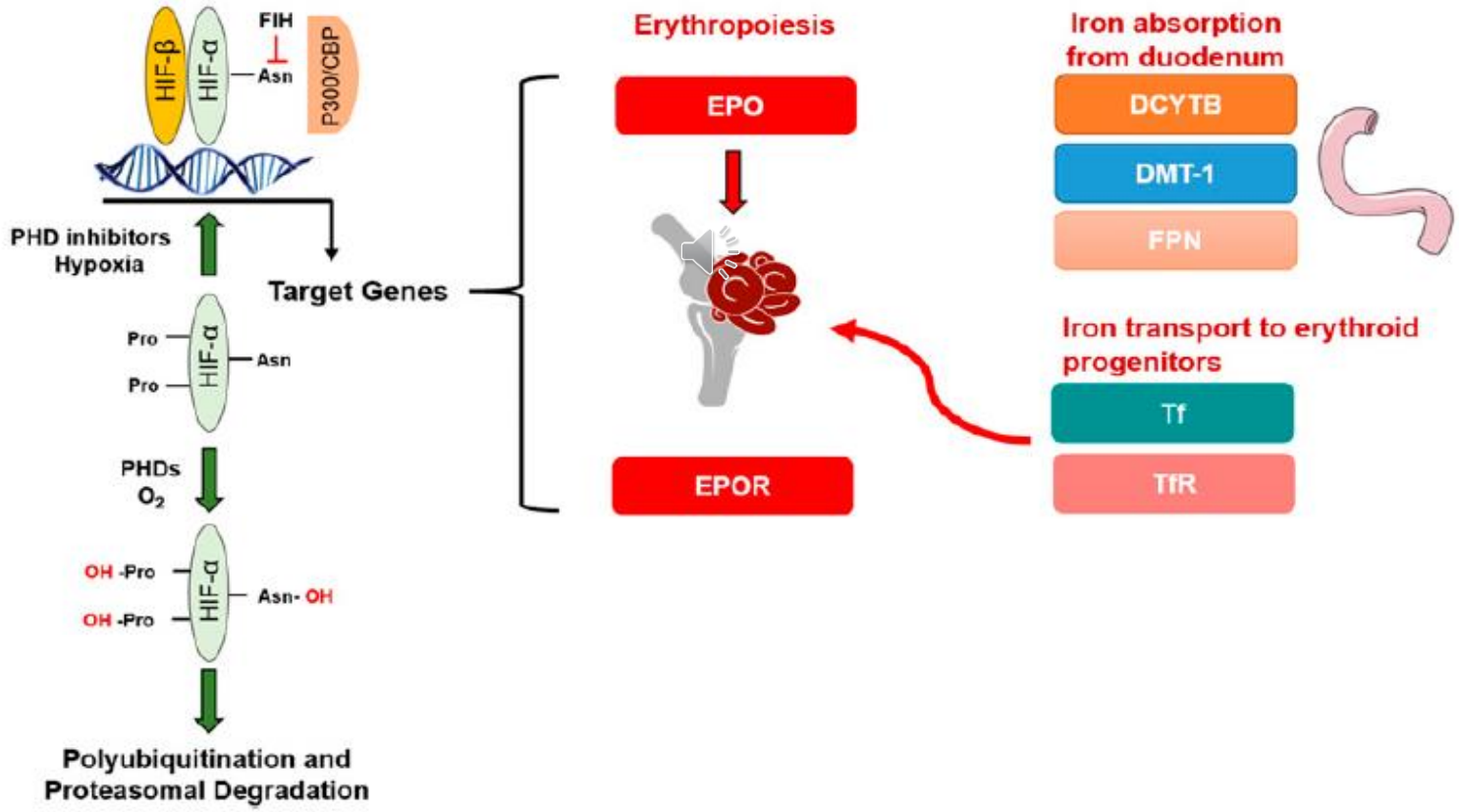
- Under stress erythropoiesis, EPO suppresses hepcidin synthesis via erythroferrone (ERFE).
- ERFE is a hormone produced by erythroblasts in response to EPO .
- HIF 1a and probably HIF2a regulates hepcidin production by directly binding to and repressing its promoter .
- While HIF-2a enhances iron availability through the activation of genes encoding DMT1 and duodenal cytochrome b (DCYTB)


Iron-sensing apparatus
Fe
TFR2/HFE
TFR1
Inflammation
IL6
Fe
Endothelial cell
BMP2/6
BMPR/HJV complex
IL6R/JAK
STAT3
SMAD
TMPRSS6
Hepcidin
Hepatocyte
Liver
FPN
Hepcidin
Duodenum
FPN
Blood loss
Erythroferrone
Fe–TF
Macrophage
FPN
Fe
HO
Ferritin
Heme
HRG
Phagolysosome
RBCs
Bone marrow
EPO
Kidney
DCYTB
HIF2
FPN
DMT1
HEPH
Fe
Ferritin
Enterocyte
↓Fe
↓O2
HIF–PHI
HIF2
EPO
Renal EPO-producing cell

IRON metabolism

- iron is transported to the liver and spleen, where it is bound to ferritin for storage, or to the bone marrow where it is used for erythropoiesis.

- the majority of iron stores are replenished by macrophage phagocytosis of the destroyed RBCs and iron recycling, a process influenced by EPO.


HIF-β
HIF-α
FIH
Asn
P300/CBP
PHD inhibitors
Hypoxia
Target Genes
Pro
Pro
HIF-α
Asn
PHDs
O_2
OH -Pro
OH -Pro
HIF-α
Asn- OH
Polyubiquitination and
Proteasomal Degradation
Erythropoiesis
EPO
EPOR
Iron absorption
from duodenum
DCYTB
DMT-1
FPN
Iron transport to erythroid
progenitors
Tf
TfR

ABSOLUTE VERSUS FUNCTIONAL IRON DEFICIENCY

- It is important to differentiate between absolute (or storage) iron deficiency and functional (or relative) iron deficiency.

- In absolute iron deficiency, the total body iron stores are depleted, limiting the production of RBCs.

➢ Contributing factors to absolute iron deficiency include :

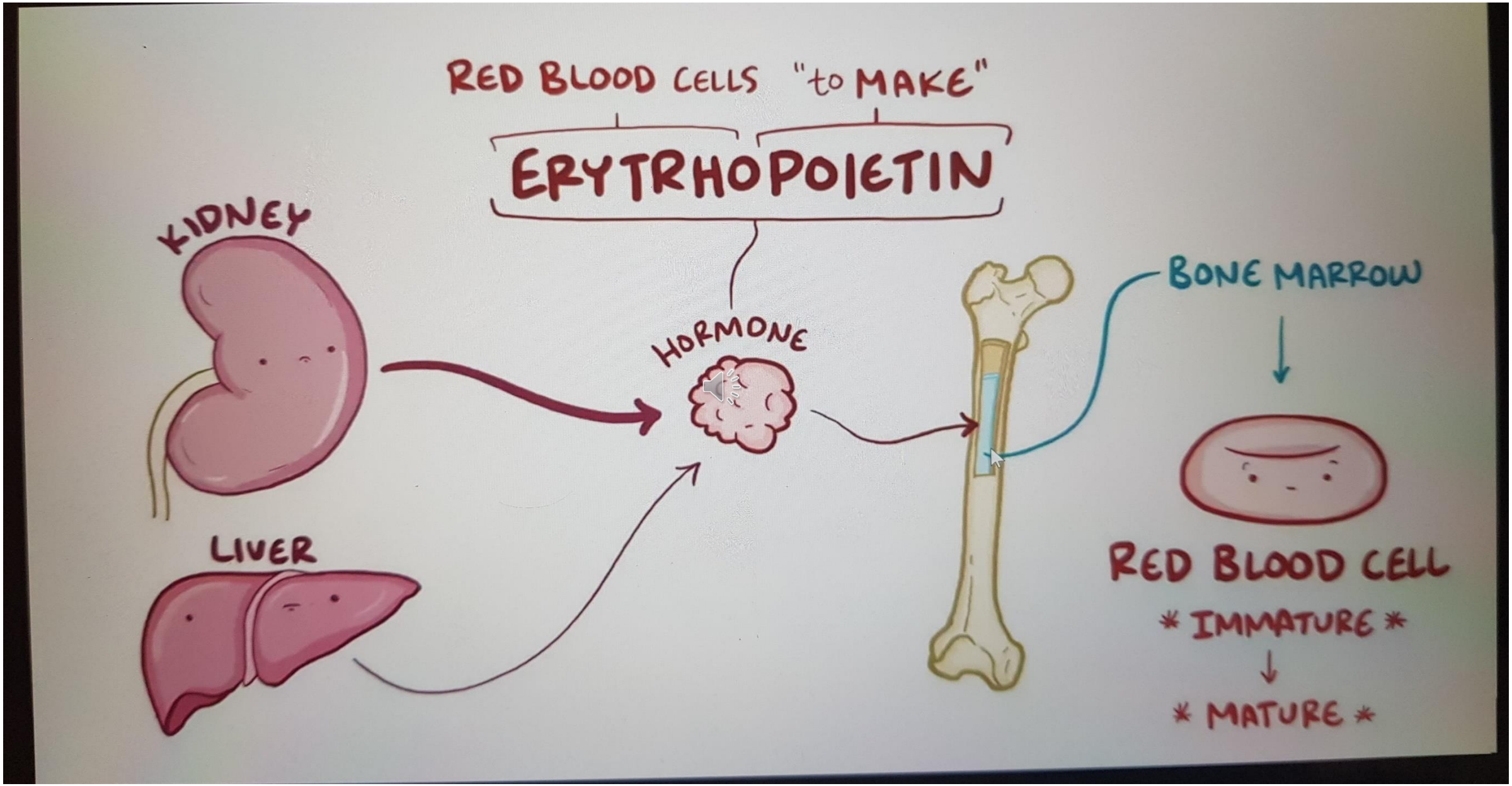
- decreased gastrointestinal absorption in patients with CKD
- and increased blood loss (for example in the setting of uremia-induced platelet dysfunction and the iatrogenic loss from serial blood draws or access-site and circuit issuesduring the dialysis procedure).

functional iron deficiency

- inefficient utilization of iron stores, stemming from one or both of two main phenomena:
- 1.anemia of chronic inflammation, is known as reticuloendothelial cell iron blockade.
- 2. to the use of exogenous EPO. Because RBC production increases in response to ESAs, the available iron may be used faster than the existing iron stores are able to release it, leading to a supply/ demand mismatch and a “relative” iron deficiency

Iron, anemia, and CKD-associated mineral and bone disorder

- Iron, inflammation, and erythropoiesis play a critical role in regulating fibroblast growth factor 23 (FGF23), which is an important contributor to CKD-MBD.
- In the absence of CKD, iron deficiency, ESA administration, and inflammation increase c-terminal FGF23 (cFGF23) levels by simultaneously increasing FGF23 transcription and cleavage.
- in CKD, where FGF23 cleavage is impaired, iron deficiency, ESAs, and inflammation increase iFGF23.
- The relative amounts of circulating iFGF23 and cFGF23 are impacted not only by iron status, inflammation, ESA use, and the presence of CKD, but also by the iron formulation administered.


RED BLOOD CELLS "to MAKE"
ERYTHROPOIETIN
KIDNEY
LIVER
HORMONE
BONE MARROW
RED BLOOD CELL
* IMMATURE *
* MATURE *