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Mitochondrial respiratory complex II and its function in cancer

Mitochondriální respirační komplex II a jeho funkce v rakovině

Doctoral thesis

Supervisor: prof. Ing. Jiří Neužil, CSc.

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List of publications

The first two publications listed here are related to this thesis.

1. **Miklovicova, S.,** Volpini, L., Sanovec, O., Monaco, F., Hadrava Vanova, K., Novak, J., Boukalova, S., Zobalova, R., Klezl, P., Tomasetti, M., Bobek, V., Fiala, V., Vcelak, J., Santarelli, L., Bielcikova, Z., Komrskova, K., Kolostova, K., Pacak, K., Dvorakova, S., Neuzil, J., 2025. Mitochondrial respiratory complex II is altered in renal carcinoma. *Biochimica Biophysica Acta (BBA) – Molecular Basis of Disease*, 1871(1):167556.

2. Sharma, P., Maklashina, E., Voehler, M., **Balintova, S.,** Dvorakova, S., Kraus, M., Hadrava Vanova, K., Nahacka, Z., Zobalova, R., Boukalova, S., Cunatova, K., Mracek, T., Ghayee, H. K., Pacak, K., Rohlena, J., Neuzil, J., Cecchini, G., Iverson, T. M., 2024. Disordered-to-ordered transitions in assembly factors allow the complex II catalytic subunit to switch binding partners. *Nature Communications*, 15:473.

3. Novak, J., Oliveira, G. L., Levoux, J., Brisudova, P., Dubisova, M., Dvorakova, S., **Miklovicova, S.,** Dalecka, M., Puttrich, V., Grycova, L., Stepanek, L., Prochazka, J., Svec, D., Magalhaes-Novais, S., Reguera, D. P., Lopez-Domenech, G., Zobalova, R., Sedlacek, R., Terp, M. G., Lansky, Z., Kittler, J., Oliveira, P. J., Ditzel, H. J., Berridge, M. V., Rodriguez, A., Rohlena, J., Nahacka, Z., Neuzil, J, 2024. The adapter protein Miro1 modulates horizontal transfer of mitochondria in cancer. *Cell Reports*. (accepted)

4. Mikyskova, R., Sapega, O., Psotka, M., Novotny, O., Hodny, Z., **Balintova, S.,** Malinak, D., Svobodova, J., Andrys, R., Rysanek, D., Musilek, K., Reinis, M., 2023. STAT3 inhibitor Stattic and its analogues inhibit STAT3 phosphorylation and modulate cytokine secretion in senescent tumour cells. *Molecular Medicine Reports*, 27(4):81.

5. Poturnajova, M., Furielova, T., **Balintova, S**., Schmidtova, S., Kucerova, L., Matuskova, M., 2021. Molecular features and gene expression signature of metastatic colorectal cancer (Review). *Oncology Reports*, 45(4):10.

Contents

Abstract

Mitochondria are found in nearly all eukaryotic cells, serving as energy producers and key regulators of metabolic pathways, such as the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS). Their role goes beyond energy metabolism, as they are important for cell signalling, redox homeostasis, and apoptosis, i.e. processes vital for cellular growth, adaptation, and survival. Central to mitochondrial function is complex II (CII), also referred to as succinate dehydrogenase (SDH), which uniquely connects the TCA cycle with the electron transport chain (ETC) by facilitating electron transfer while regulating succinate and fumarate, metabolites with important signalling effects. Alterations in CII, both in its structure, function and assembly, have been increasingly implicated in various cancers.

Renal cell carcinoma (RCC) and pheochromocytoma/paraganglioma (PPGL) are two types of cancer where mitochondrial dysfunction, particularly involving CII, has emerged as a distinctive hallmark. RCC, notably in its clear cell subtype (ccRCC), exhibits unique metabolic reprogramming that contributes to its aggressive phenotype and resistance to conventional treatments. PPGLs, while less common, present distinct metabolic challenges in the clinical setting due to frequent genetic mutations impacting mitochondrial function. These types of cancer share similarities in their dependence on mitochondrial metabolism, but they also show unique mitochondrial alterations that may inform subtype-specific therapies.

In this study, we explore the role of CII in the metabolic changes of RCC subtypes. Using patient tumour samples, this work reveals that ccRCC tumours display significantly reduced mitochondrial DNA (mtDNA), protein content, and CII activity, all of which support a shift to glycolysis, a hallmark adaptation in ccRCC that fuels its proliferation under hypoxic conditions. In contrast, papillary (pRCC) and chromophobe (chRCC) RCC subtypes retain mitochondrial features similar to healthy kidney tissue. This subtype-specific mitochondrial profile emphasizes the potential of CII as both a biomarker and a therapeutic target in RCC, as its impaired function is linked to the aggressive behaviour of ccRCC.

Moreover, our further investigation complements these findings by providing deeper insights into the details of CII assembly. Using a PPGL cell line, this research has identified the critical

roles of SDHAF2 and SDHAF4 assembly factors in SDHA subunit maturation. In knockout models, loss of SDHAF4 leads to abnormal CII assembly, reduced succinate dehydrogenase activity, and excessive succinate accumulation. This mechanistic insight into CII assembly is crucial for understanding how defects in assembly factor function could drive mitochondrial and metabolic abnormalities in tumours with CII dysfunction.

Together, these studies enhance our understanding of the diverse roles of CII in cancer metabolism and assembly, revealing CII as a key metabolic regulator. By linking structural and functional aberrations in CII to specific cancer subtypes, this research will support the development and deeper understanding of mitochondrial-targeted therapies, such as the mitochondria-specific agent MitoTam. As mitochondrial dysfunction and metabolic reprogramming remain central points in cancer biology, the findings of these studies contribute valuable insights into therapeutic strategies for targeting mitochondrial integrity and metabolism in renal and adrenal cancers.

Key words

Complex II, succinate dehydrogenase, renal cancer, pheochromocytoma and paraganglioma, mitochondria, CII_{low}

Abstrakt

Mitochondrie se nacházejí téměr ve všech eukaryotických buňkách, kde produkují energii a regulují důležité metabolické procesy, jako je Krebsův cyklus (TCA) a oxidativní fosforylace (OXPHOS). Mitochondrie hrají také roli v buněčné signalizaci, redoxní rovnováze a apoptóze, což jsou procesy důležité pro růst, adaptaci a přežití buněk. Komplex II (CII), neboli sukcinátdehydrogenáza (SDH), propojuje TCA cyklus s ETC řetězcem, a tak participuje na přenosu elektronů a regulaci metabolitů, jakými jsou sukcinát a fumarát, které mají důležité signální účinky. Změny v CII, ať už v jeho struktuře, funkci nebo asemblaci, jsou stále častěji spojovány s různými typy rakoviny.

Renální karcinom (RCC) a feochromocytom/paragangliom (PPGL) jsou nádorové patologie, u nichž je významným znakem mitochondriální dysfunkce, zejména spojená s CII. RCC, především podtyp světlobuněčnému karcinomu (ccRCC), vykazuje výrazné metabolické změny, které přispívají k jeho agresivnímu chování a rezistenci vůči běžným léčbám. PPGL, byť vzácnější, představuje specifické metabolické výzvy kvůli častým genetickým mutacím, které ovlivňují mitochondriální funkci. Obě tyto nádorové patologie jsou závislé na mitochondriálním metabolismu, a také vykazují unikátní mitochondriální změny, které mohou být využité k vývoji cílených terapií zaměřených na specifické podtypy nádorů.

V této studii zkoumáme roli CII v metabolických změnách podtypů RCC. Výzkum na vzorcích nádorů od pacientů ukazuje, že nádory ccRCC mají výrazně snížený obsah mitochondriální DNA (mtDNA) a proteinů a sníženou aktivitu CII. Tyto nádory využívají glykolýzu na růst v hypoxických podmínkách. Naproti tomu podtypy papilárního (pRCC) a chromofobního (chRCC) RCC si zachovávají mitochondriální vlastnosti podobné zdravé ledvinové tkáni. Tento specifický mitochondriální profil jednotlivých RCC podtypů poukazuje na potenciál CII jako biomarkeru a terapeutického cíle v RCC, protože jeho narušená funkce je spojena s agresivním chováním tohoto nádorového onemocnění.

Náš další výzkum rozšiřuje tato zjištění tím, že poskytuje hlubší pohled na mechanismus asemblace CII. Studie za použití buněčné linii PPGL ukazují důležitou roli asemblačních faktorů SDHAF2 a SDHAF4 při maturaci podjednotky SDHA. V modelech, kde došlo k vyřazení SDHAF4, byla pozorována tvorba alternativních forem CII, snížená aktivita enzyme SDH a

nadměrné hromadění sukcinátu. Tento mechanistický pohled na asemblaci CII je klíčový pro pochopení toho, jak poruchy ve funkcích těchto asemblačních faktorů mohou způsobit mitochondriální a metabolické abnormality u nádorů s dysfunkcí CII.

Tento výzkum přispívá k lepšímu pochopení různých rolí CII v metabolizmu nádorových onemocnění, ukazující CII nejen jako důležitý metabolický regulátor, ale také jako strukturu náchylnou na dysfunkci v nádorových onemocněních. Spojením strukturálních a funkčních abnormalit CII s konkrétními typy rakoviny tento výzkum může přispět k rozvoji a lepšímu pochopení terapií zaměřených na mitochondrie, například u mitochondriálně cíleného léku MitoTam. Vzhledem k tomu, že mitochondriální dysfunkce a metabolické změny zůstávají klíčovými znaky v biologii rakoviny, výsledky této studie poskytují cenné informace pro rozvoj terapeutických strategií zaměřených na mitochondriální integritu a metabolismus u rakoviny.

Klíčová slova

Komplex II, sukcinátdehydrogenáza, renální karcinom, feochromocytom a paragangliom, mitochondrie, CII_{low}

List of abbreviations

αKG – α-keto glutarate

ATP – adenosine triphosphate

BN-PAGE – blue native polyacrylamide gel electrophoresis

ccRCC – clear cell renal carcinoma

CAIX – carbonic anhydrase

CI – complex I

CII – complex II

CIII – complex III

CIV – complex IV

CV – complex V

chRCC – chromophobe renal carcinoma

CoQ – coenzyme Q, oxidized

$CoQH_2$ – coenzyme Q, reduced

DHODH – dihydroorotate dehydrogenase

FAD – flavin adenine dinucleotide

$FADH_2$ – flavine adenine dinucleotide, reduced

FMN – flavin mononucleotide

GOT1 – glutamate-oxaloacetate transaminase 1

GOT2 – glutamate-oxaloacetate transaminase 2

HIF – hypoxia-inducible factor

HK-2 – hexokinase-2

IBM – inner boundary membrane

IL-β – interleukin β

IMM – inner mitochondrial membrane

KO – knockout

MitoTam – mitochondrially-targeted tamoxifen

mtDNA – mitochondrial DNA

NAD – nicotinamide adenine dinucleotide

NDUFA4L2 – NADH oxidoreductase subunit A4-like 2

OAA – oxaloacetate

OMM – outer mitochondrial membrane

OXPHOS – oxidative phosphorylation

PARP – poly(ADP-ribose) polymerase

PCC – pheochromocytoma

PDH – pyruvate dehydrogenase

PE – phosphatidylethanolamine

PFKP – phosphofructo kinase platelet

PHD – prolyl hydroxylase domain

PKM2 – pyruvatekinase M2

PPGL – paraganglioma

pRCC – papillary renal carcinoma

RCC – renal cell carcinoma

RET – reverse electron transfer

ROS – reactive oxygen species

SC – supercomplex

SDH – succinate dehydrogenase

SQR – succinate quinone reductase

SUCNR1 – succinate receptor 1

TCA – tricarboxylic acid cycle

TFAM – transcription factor A, mitochondrial

TKI – tyrosine kinase inhibitor

TPP – triphenyl phosphonium

VDAC – voltage-dependent anion channel

VEGF – vascular endothelial growth factor

VHL – von-Hippel Lindau

1. Introduction

1.1. Mitochondria

Mitochondria are highly specialized organelles that act as main energy producers within the cell, generating the majority of ATP through oxidative phosphorylation (OXPHOS). This process is essential for cellular function, particularly in tissues with high energy demands like the brain, heart, and kidneys. However, mitochondria have a range of functions beyond ATP synthesis; they are central to cellular homeostasis, supporting key metabolic pathways such as fatty acid oxidation, amino acid metabolism and the tricarboxylic acid (TCA) cycle. These metabolic activities help regulate nutrient processing and adapt energy production to meet the dynamic needs of the cell [1]. In addition to their metabolic role, mitochondria influence cell signalling and its regulation. They are significant sources of reactive oxygen species (ROS), which serve as signalling molecules in pathways that control cellular processes such as proliferation, differentiation, and response to stress [2]. Moreover, mitochondria are critical in regulating apoptosis, a process that removes damaged or dysfunctional cells and helps prevent cancer development [3].

1.1.1. Mitochondrial genome organization

A defining feature that sets mitochondria apart from other organelles is their unique mitochondrial genome. Mitochondrial DNA (mtDNA) encodes 13 essential polypeptides, which are core components of the respiratory chain complexes that drive OXPHOS. These proteins are integrated into three complexes of the electron transport chain (ETC) and ATP synthase: 7 for complex I (CI), 1 for complex III (CIII), 3 for complex IV (CIV), and 2 for complex V (CV). The remaining approximately 80 subunits of the OXPHOS system are encoded by the nuclear genome [4].

1.1.2. Mitochondrial structure

Mitochondria are double-membraned, containing the outer membrane (OMM) and the inner membrane (IMM). The OMM contains porins that allow the passage of peptides, metabolites,

and ions. The most abundant protein within the OMM is voltage-dependent anion channel (VDAC). In contrast, the IMM is one of the most protein-dense membranes in the cell, organizing enzymes to optimize the efficiency of metabolic process by positioning then close to each other. It is structurally divided into the inner boundary membrane (IBM), which lies parallel to the OMM, and the cristae membranes, which extend inward and form invaginations known as cristae [5]. These cristae structures maximize the surface area of the IMM for the proper organization and function of OXPHOS complexes. Cristae are connected to the IBM through narrow tubular or slit-like cristae junctions, increasing the surface area of the IMM. The IMM's structure is highly dynamic and can change in response to cellular needs, redox status, or apoptotic signals, adjusting the organization and density of cristae accordingly. This flexible architecture is vital for maintaining energy production and signalling [6]. Even though the intermembrane space (IMS) is the smallest sub-compartment of mitochondria, it is involved in protein transport, lipid homeostasis and apoptosis. The matrix contains the mitochondrial genome and regulates calcium signalling. Importantly, matrix is a platform for numerous metabolic processes, such as the TCA cycle [7].

1.1.3. Tricarboxylic acid (TCA) cycle

The TCA cycle, also known as the Krebs cycle or citric acid cycle is a series of metabolic reactions that generate energy by means of oxidation of acetyl-CoA, derived from carbohydrates, fatty acids, and proteins. The TCA cycle begins when acetyl-CoA combines with oxaloacetate (OAA) to form citrate, a reaction catalyzed by citrate synthase (CS). Citrate is then converted to isocitrate by aconitase 2. Isocitrate undergoes decarboxylation, catalyzed by isocitrate dehydrogenase 3 or isocitrate dehydrogenase 2, resulting in the formation of α-ketoglutarate (αKG) and the release of CO_2. αKG is further decarboxylated to succinyl-CoA by the oxoglutarate dehydrogenase complex, producing NADH and releasing another molecule of CO_2. Succinyl-CoA is converted to succinate by succinyl-CoA synthetase, coupled with the generation of GTP or ATP, depending on the energy needs of the tissue. Succinate is then oxidized to fumarate by succinate dehydrogenase (SDH), followed by conversion of fumarate to malate by fumarate hydratase. Finally, malate is converted back to OAA by malate dehydrogenase 2, completing the cycle. Each turn of the TCA cycle produces three NADH and one $FADH_2$ molecules, which are crucial for transferring electrons to the ETC, supporting the

production of ATP [8].

In certain cellular contexts, such as in cancer cells with defective mitochondria, or those exposed to hypoxia, the TCA cycle can function in reverse. In this case, αKG is reductively carboxylated to form citrate, supporting the synthesis of acetyl-CoA necessary for lipid biosynthesis and cell viability under conditions of limited oxygen or metabolic stress [9,10]. A schematic of the forward and reverse TCA cycle is illustrated in Figure 1.

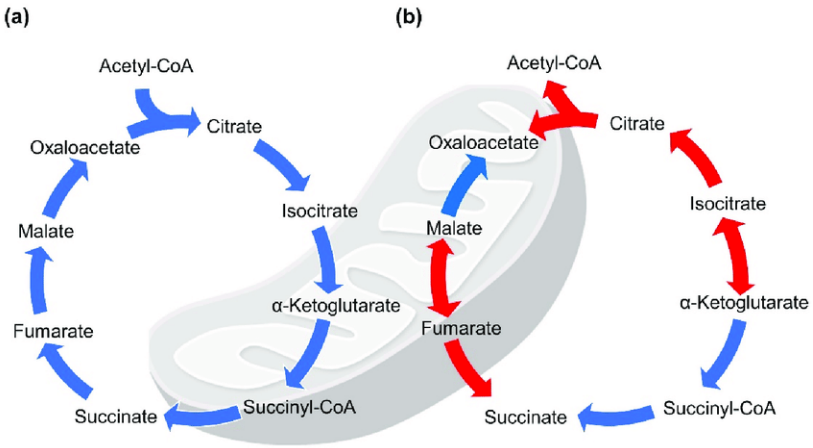

Figure 1: Overview of the forward and reverse TCA cycle (reductive carboxylation). Adapted from [11].

1.1.4. Electron transport chain and oxidative phosphorylation

The OXPHOS system generates ATP through a series of protein complexes known as the ETC, which is embedded in the IMM. The ETC comprises four protein complexes (I-IV) and two electron carriers, cytochrome c and coenzyme Q (CoQ), which facilitate the transfer of electrons from NADH and $FADH_2$ to oxygen, the final electron acceptor, producing water as a byproduct. As electrons pass through these complexes, they drive the pumping of protons across the IMM, creating a proton gradient. This gradient powers ATP synthase (CV), which uses the energy from the proton flow to synthesize ATP [12].

CI (NADH-ubiquinone oxidoreductase) is the largest enzyme complex in the ETC, composed of 14 core subunits arranged into two functional domains: the membrane arm, responsible for

proton translocation, and the peripheral arm, involved in electron transfer [13,14]. In humans, CI is composed of 45 polypeptides, reflecting its intricate structure and central role in cellular respiration. The enzyme catalyzes the transfer of electrons from NADH, produced in the TCA cycle, to ubiquinone (CoQ), facilitated by iron-sulfur (Fe-S) clusters and flavin mononucleotide (FMN) in the matrix arm. This electron transfer triggers conformational changes within CI that promote the translocation of protons across the IMM, contributing to the proton gradient required for ATP synthesis [15,16].

CII (succinate dehydrogenase; SDH), is distinct among ETC components due to its dual role in cellular metabolism: it functions both within the TCA cycle and in electron transfer to the ETC. CII catalyzes the oxidation of succinate to fumarate while transferring electrons to CoQ. Unlike other ETC complexes, it does not directly contribute to the proton gradient across the IMM [17].

CIII (CoQ-cytochrome c oxidoreductase), is a key component of the ETC, transferring electrons from reduced ubiquinone (ubiquinol; $CoQH_2$) to cytochrome c, a soluble electron carrier in the IMS. This process occurs via the Q-cycle, enabling both electron transfer and proton pumping. Protons translocated into the IMS contribute to the proton motive force that drives ATP synthesis by ATP synthase. CIII functions as a dimer, with 11 subunits in each monomer. While it does not directly accept electrons from CI or CII, it is fed by CoQ reduced by these complexes and other dehydrogenases from various metabolic pathways [18,19].

CIV (cytochrome c oxidase), serves as the final enzyme in the ETC, catalyzing electron transfer from cytochrome c to molecular oxygen, which is reduced to water. This complex is composed of 13 subunits. The electron transfer process is coupled with proton translocation across the IMM: four protons are used to form water, while additional four protons are pumped into the IMS, reinforcing the proton gradient essential for ATP synthesis [20].

CV (ATP synthase), uses the proton gradient created by the ETC to produce ATP. It comprises two main domains: the F_1 domain, situated in the matrix, and the F_0 domain, embedded in the IMM. The enzyme operates by coupling proton flow through the F_0 domain to the rotational

motion, triggering conformational changes in the F_1 catalytic core. These structural shifts enable the phosphorylation of ADP into ATP, finalizing the process of mitochondrial energy generation [21]. A schematic of the OXPHOS and TCA cycle, with CII being at the crossroads, is illustrated in Figure 2.

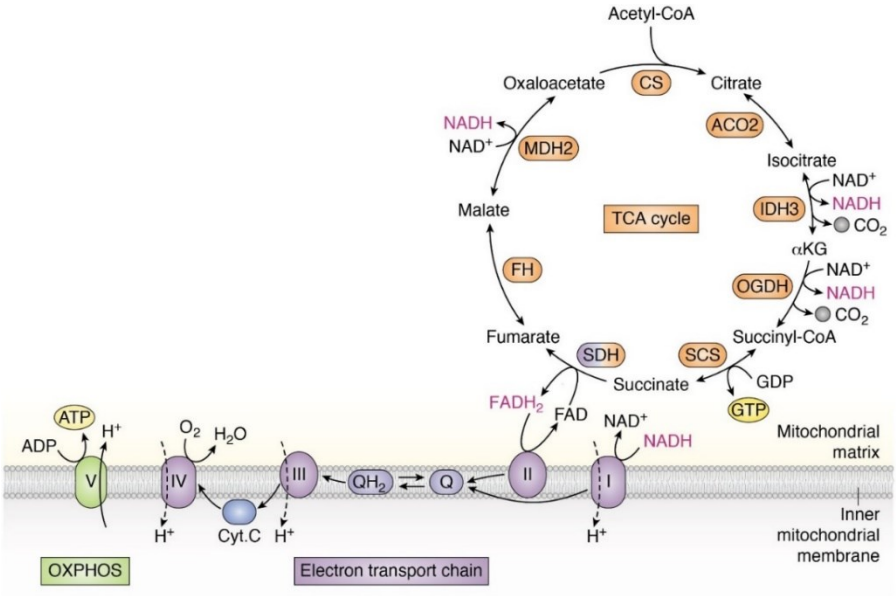

Figure 2: Overview of OXPHOS and TCA cycle. The figure shows CII as a central element bridging the OXPHOS and TCA cycle. Adapted from [22].

The organization of the ETC complexes has long been debated, with early models proposing either a fixed "solid-state" arrangement [23] or a more dynamic "fluid-state" model where complexes diffuse across the IMM [24]. Recent advances show that ETC complexes assemble into larger structures known as supercomplexes (SCs). According to the most recent “plasticity” model, free ETC complexes coexist with SCs, allowing a flexible assembly that allows dynamic adaptation according to cellular needs [25].

Supercomplex assembly provides a kinetic advantage, boosts ATP production and influences the IMM morphology in forming cristae. Respirasomes are structural SCs involved in complete respiration from NADH to oxygen. They consist of the minimal functional unit comprising CI-$CIII_2$-CIV_n,. These SCs thought to improve electron transfer between ETC complexes and optimize the proton motive force needed for ATP synthesis [26–28].

Variants of this structure include more copies of CIV, although they represent a minor fraction of CI-containing SCs. Other notable configurations include a CI + $CIII_2$ complex, while a CI_2 + $CIII_2$ + CIV_2 "megacomplex" has been observed previously [29]. Although CII has not been definitively linked to human SC assembly [30], findings in ciliates suggest the possibility of CI + CII + $CIII_2$ + CIV_2 SCs, potentially contributing to the IMM bending [31].

1.2. Complex II biology

1.2.1. Structure and assembly

CII stands out among mitochondrial complexes for its relatively simple composition. It comprises four subunits: the hydrophilic SDHA and SDHB, and the membrane-bound hydrophobic SDHC and SDHD, all of which are encoded by nuclear DNA. Exceptions to this nuclear encoding are found in certain red algae and heterotrophic zooflagellates, where the SDHB, SDHC, and SDHD subunits are encoded by mtDNA [32]. Following synthesis on cytosolic ribosomes, the subunits are imported into the mitochondrial matrix via the TIM/TOM protein import system. Inside the matrix, the subunits undergo folding, insertion of cofactors, and stepwise assembly into the functional SDH holoenzyme [33]. The assembly process involves three key stages: maturation of SDHA, maturation of SDHB, coordination of heme between SDHC and SDHD, and finally integration of all subunits into the complete CII complex. The structure of CII is illustrated in Figure 3.

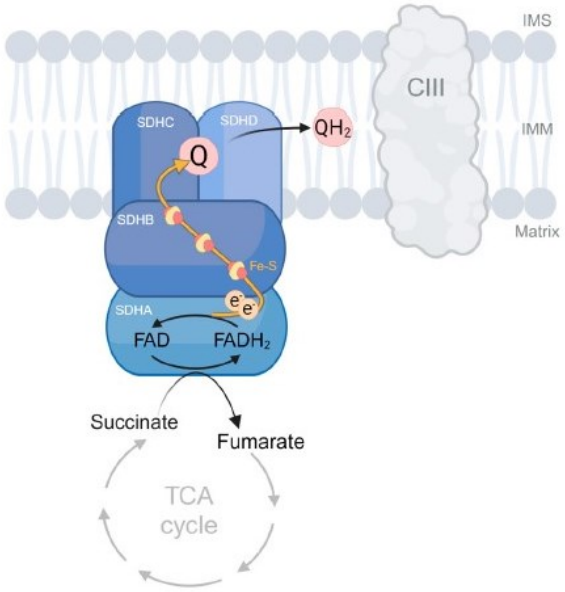

Figure 3: Structure of CII. Adapted from [34].

1.2.1.1. SDHA maturation

SDHA, the largest subunit of CII, is a flavoprotein composed of four distinct domains: the flavin adenine dinucleotide (FAD)-binding domain, the capping domain, the helical domain, and the C-terminal domain [35]. Under aerobic conditions, SDHA facilitates the oxidation of succinate, during which the FAD cofactor is reduced to $FADH_2$. Electrons from $FADH_2$ are subsequently transferred via Fe-S clusters in the SDHB subunit, ultimately reducing CoQ in the ETC [36].

While many flavoproteins incorporate FAD through an autocatalytic process without the need for additional factors [37], the integration of FAD into SDHA in humans requires the SDHAF2 assembly factor. This process, known as flavinylation, involves the covalent binding of FAD to SDHA. The role of SDHAF2 in this mechanism remains a subject of debate. Some studies suggest that SDHAF2 acts as a chaperone, restructuring SDHA to facilitate FAD binding. However, when SDHAF2 levels are low, SDHA can bind FAD autocatalytically, although the kinetics of this reaction is slow and inefficient [38,39]. In contrast, others propose that SDHAF2 directly binds to FAD and functions as a transporter, delivering FAD to SDHA [37,40].

Interestingly, some thermophilic archaea [41] and mammalian breast cancer cells [42] do not

require SDHAF2 for flavinylation. This raises questions about the evolutionary advantage of developing an assembly factor like SDHAF2 if it is not essential for forming a functional CII. However, SDHAF2 plays a crucial role in enhancing the stability of the SDHA-FAD complex and accelerating the flavinylation process [43].

Recent studies have revealed additional roles for SDHAF2 beyond its involvement in CII assembly. It has been shown to contribute to mitochondrial respiration by stabilizing both CII and CIV assemblies. Notably, in HeLa cells, SDHAF2 is essential for COX assembly, as its removal leads to reduced COX activity without affecting the expression of SDH subunits, suggesting that SDHAF2 may influence COX assembly either through direct interaction with subunits or by modifying the mitochondrial ultrastructure [44]. Furthermore, SDHAF2 overexpression has been linked to enhanced mitochondrial function and lipid oxidation, while knockdown of the fission protein Drp1 in muscle cells results in reduced CII assembly and activity. Restoration of SDHAF2 in Drp1-deficient cells restored CII activity and improved lipid oxidation and insulin response. This highlights the role of Drp1 as a chaperone for SDHAF2, facilitating its proper localization in mitochondria for CII assembly [45].

Another important assembly factor in SDHA maturation is SDHAF4, which primarily protects the SDHA subunit when it is not bound to SDHB. During the earlier step of SDHA in succinate oxidation and the reduction of FAD to $FADH_2$, excess electrons can result in superoxide production if they are not efficiently transferred to SDHB. SDHAF4 binds directly to the SDHA-FAD complex as a chaperone, which prevents oxidative stress triggered by FAD and subsequent ROS production [36,40]. While other functions of SDHAF4 remain unexplored, its absence has been shown to trigger mitophagy, cause dilated cardiomyopathy, and neurodegeneration, indicating its involvement in diverse pathological conditions [46,47].

1.2.1.2. SDHB maturation

SDHB is located between SDHA and the SDHC-SDHD dimer within CII and contains two domains with three [Fe–S] clusters. These clusters, comprising a [2Fe–2S] cluster at the N-terminal, and [3Fe–4S] and [4Fe–4S] clusters at the C-terminal, are essential for transferring

electrons from succinate oxidation to CoQ. The synthesis of these Fe-S clusters involves the scaffold protein ISCU, through a complex composed of a cysteine desulfurase, NFS1, its binding partner, ISD11, and an iron donor, frataxin [48]. The [2Fe-2S] cluster is formed through the Grx5 complex [49]. Once synthesized, the Fe-S clusters are transferred to a scaffold complex consisting of HSC20, HSPA9 and ISCU before being incorporated into SDHB. Together, SDHA and SDHB form the catalytic heterodimer of CII, stabilized by FAD and the [Fe–S] clusters [50].

Two assembly factors, SDHAF1 and SDHAF3, are involved in SDHB maturation. SDHAF1 appears to facilitate Fe–S cluster insertion by binding SDHB at non-LYR-binding sites and by guiding the HSPA9-HSC20-ISCU complex towards the LYR-binding sites on SDHB [51,52]. These Fe–S clusters are then transferred to SDHB, and due to their susceptibility to oxidative damage, SDHAF1 and SDHAF3 protect them during this process [49]. SDHAF3 is proposed to facilitate the binding of SDHAF1 to SDHB, enhancing the transfer and incorporation of Fe–S clusters into SDHB [53,54].

1.2.1.3. Assembly of CII

The ubiquinone-binding domain is located at the interface of the SDHC and SDHD transmembrane helices and the C-terminal region of SDHB. Electrons generated from succinate oxidation are sequentially transferred through a series of redox centers, including FAD, [2Fe-2S], [3Fe-4S], and [4Fe-4S] clusters. CoQ is the primary electron acceptor, although electrons from the [3Fe-4S] cluster can be passed to either heme b or CoQ [55]. While heme b is not essential for quinone reduction, it may function as an electron reservoir in the transfer pathway. The electron transfer process begins with the reduction of CoQ by the first electron from the [3Fe-4S] cluster to form semiquinone radical (CoQH), which is then stabilized by electron equilibration between heme b and CoQ, before accepting a second electron to complete the reduction [56].

Following the dissociation of SDHAF1 and SDHAF3, the hydrophilic portion of the SDHA-SDHB intermediate interacts with the SDHC-SDHD dimer [12]. This SDHC-SDHD dimer, containing both heme b and two CoQ binding sites, functions as the terminal electron transfer

point from CII to CoQ [55]. Additionally, a phosphatidylethanolamine (PE) molecule within the dimer stabilizes the protein structure. Assembly factors such as Coa1, Coa2, and Shy1 play a role in incorporating heme b into the membrane anchor [43]. A schematic of the CII assembly is illustrated in Figure 4.

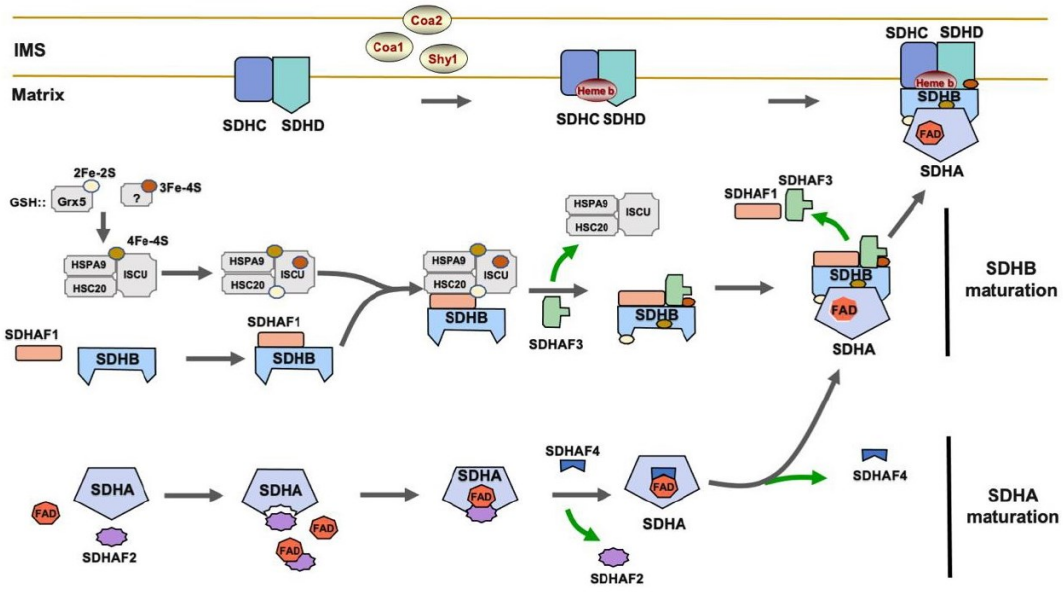

Figure 4: Overview of CII assembly. Adapted from [57].

1.2.1.4. Chaperones

HSP60 is a chaperone protein essential for ETC chain complex assembly, including proper folding of SDHA [58]. In addition, TRAP1, which has been shown to regulate mitochondrial metabolism [59], has been identified as an SDHB chaperone [60,61]. While one study demonstrated that TRAP1 inhibits CII, thereby contributing to tumorigenesis, another study found that TRAP1 maintains CII, preserving mitochondrial homeostasis [62,63].

1.2.2. Regulation of CII activity

The catalytic activity of CII is regulated by various post-translational modifications, including phosphorylation, deacetylation, and succinylation. While the impact of phosphorylation on SDH activity is still unclear, studies have shown that deacetylation of SDHA enhances CII activity [64]. Succinylation, which can occur both enzymatically and nonenzymatically, has also been identified as a regulatory mechanism. Six succinylation sites on SDHA and one on

SDHB have been found to enhance CII activity and mitochondrial respiration [65].

1.2.3. Reverse electron transfer

Reverse electron transfer (RET) in CII has been observed in recent studies [66,67]. In this reversed activity, CII reduces fumarate to succinate, making fumarate an alternative electron acceptor to oxygen within the ETC. Fumarate reduction helps maintain the flow of electrons into the ETC through CI and dihydroorotate dehydrogenase (DHODH), supporting NADH oxidation and promoting de novo pyrimidine biosynthesis during hypoxia [67]. Additionally, reverse CII activity may contribute to the anaerobic production of hydrogen in mitochondria [68].

1.2.4. Generation of reactive oxygen species

While CI and CIII are typically considered the primary sources of ROS, such as superoxide, CII has not been explicitly associated with significant ROS production. However, recent studies have identified pathways through which CII contributes to ROS generation. The first is indirectly via RET. When succinate is high in cells, CII overproduces QH_2 and electrons from QH_2 are redirected to the FMN site of CI. A reaction between O_2 and reduced FMN leads to the production of superoxide [69]. Furthermore, ROS production at the Q site of CII has also been reported, though this mechanism is considered to be rare in mammals [70]. Superoxide produced through these pathways is subsequently converted to hydrogen peroxide (H_2O_2) and hydroxyl radical (OH). This can lead to oxidative stress and contribute to cellular damage. Prolonged exposure to ROS can result in the oxidative modification of mitochondrial and cellular components, including proteins, lipids, and nucleic acids, ultimately leading to genomic instability and cellular dysfunction [71–73].

1.2.5. Succinate signalling

Succinate, an intermediate in the TCA cycle, becomes a potent oncometabolite when it accumulates, particularly under conditions of CII deficiency. While it normally resides within the mitochondrial matrix, succinate can translocate to the cytoplasm and extracellular space during pathological states such as cancer. This translocation is facilitated by mitochondrial

membrane transporters, including the solute carrier family 25 member 10 (SLC25A10) and porins, which enable its movement across the IMM and OMM membranes, respectively [74].

Extracellular succinate activates the succinate receptor-1 (SUCNR1), initiating downstream signalling pathways like STAT3 and ERK1/2. This results in the stimulation of vascular endothelial growth factor (VEGF), triggering angiogenesis [75]. Moreover, SUCNR1 activation stabilizes hypoxia-inducible factors (HIFs), which induces interleukin-1β (IL-1β) overexpression, driving the M1 polarization of macrophages and enhancing tumour angiogenesis [76,77]. Additionally, the uptake of succinate in T-cells can suppress their effector function [78].

Intracellular succinate, structurally similar to α-KG, acts as a competitive inhibitor of α-KG-dependent enzymes, including prolyl hydroxylase (PHD), which are critical for protein hydroxylation, DNA and histone demethylation [79]. By inhibiting PHD, succinate stabilizes HIF-1α, leading to transcriptional activation of genes involved in glycolysis and angiogenesis [80,81]. Moreover, succinate inhibits the TET family of 5'-methylcytosine hydroxylases and Jumonji domain-containing histone demethylases (JMHD), causing global histone hypermethylation and altering gene expression regulation [82]. A schematic of the succinate signalling is illustrated in Figure 5.

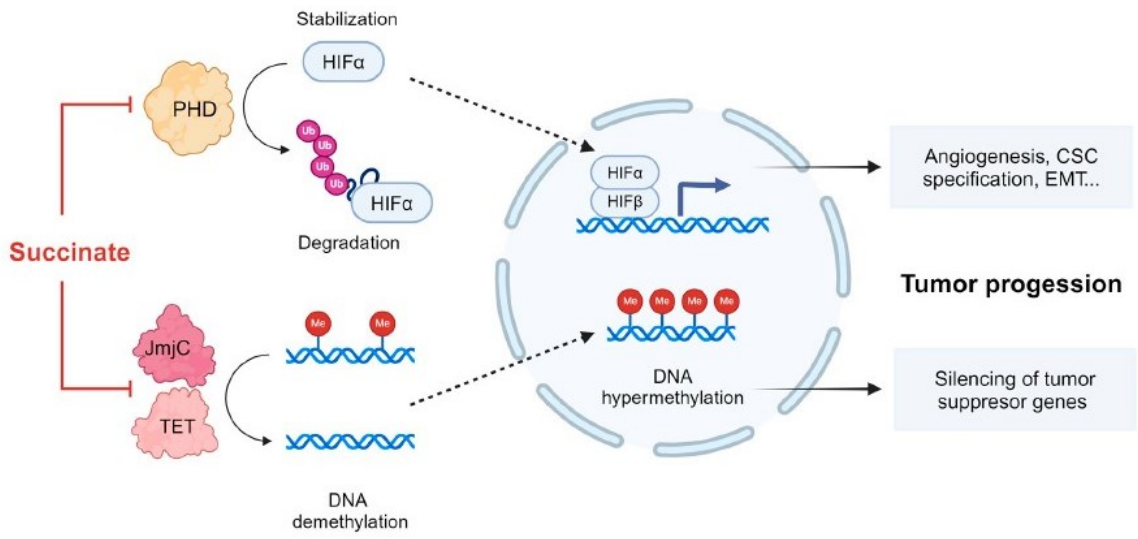

Figure 5: Overview of succinate signalling. Adapted from [34].

Interestingly, succinate accumulation in models of CII inhibition has been shown to impair homologous recombination (HR)-mediated DNA repair. These HR defects make tumour cells more susceptible to synthetic lethality when targeted with poly(ADP)-ribose polymerase (PARP) inhibitors [83,84]. This highlights the complex role of succinate in both promoting tumourigenesis and offering potential therapeutic opportunities in cancer treatment.

1.3. Complex II in disease

Mutations in the genes encoding components of CII can cause mitochondrial dysfunction, which can manifest as a primary mitochondrial disease due to inherited germline mutations, or as an increased risk of tumour development arising from somatic mutations.

1.3.1. Non-cancerous diseases

Mitochondrial CII deficiency accounts for approximately 2% of all diagnosed mitochondrial diseases, making it a rare inherited metabolic disorder [85]. Clinical presentation of CII deficiency varies depending on which subunit carries the mutation. Since SDHA is the primary catalytic subunit of CII and is the most stable of the CII subunits, mutations in SDHA are rare and their clinical manifestation depends on the degree of CII activity loss in specific tissues [86,87]. Mutations in the SDHA gene most frequently lead to Leigh syndrome, a neurodegenerative disorder characterized by progressive loss of mental and movement abilities and respiratory failure [88]. The frequency and clinical outcome of individual mutations vary. Certain mutations may only result in cardiomyopathy, while others may present with both neurological and cardiac manifestations. In some cases, dilated cardiomyopathy may occur without any neurological involvement [89].

There is also increasing evidence suggesting that dysfunction of CII contributes to age-related diseases [90,91]. Specifically, reduced CII activity has been observed in several neurodegenerative conditions, namely Alzheimer's disease [92] and Parkinson's disease [93]. Surprisingly, SDH deficiency has been associated with unusual phenotypes, including obesity [94] and premature aging of hematopoietic stem cells [95].

1.3.2. Cancer

CII deficiency has been most commonly linked to cancers such as pheochromocytoma/paraganglioma (PCC/PPGL), renal cell carcinoma (RCC), and gastrointestinal stromal tumours (GIST). Epigenetic changes in the SDHC gene are associated with the Carney triad, a rare, non-hereditary syndrome that includes SDH-deficient GIST, SDH-deficient paraganglioma, and pulmonary chondroma [96].

Although less common, SDHB mutations have been implicated in other malignancies, including multiple hamartomas, gastric cancer [97], T-cell acute leukemia [98], and neuroblastoma [99].

Gastrointestinal stromal tumours (GIST) are the most prevalent mesenchymal tumours of the gastrointestinal tract. Approximately half of wild-type GIST cases, which lack mutations in the KIT or PDGFRA genes, are SDH-deficient [100–102] and are often associated with global DNA hypermethylation [103].

1.3.2.1. Pheochromocytoma/paraganglioma

Pheochromocytomas (PCCs) and paragangliomas (PPGLs) are catecholamine-secreting neuroendocrine tumours originating from chromaffin cells, which are derived from the neural crest. Traditionally, PCCs were distinguished as adrenal medulla tumours, while PPGLs were classified as extra-adrenal tumours arising in the sympathetic nervous system (typically in the pelvis and abdomen) or the parasympathetic nervous system (commonly in the head, neck and rarely in the thorax and pelvis). However, a recent WHO classification of endocrine and neuroendocrine tumours re-defined PCC as an intra-adrenal form of PPGL originating from chromaffin cells of the adrenal medulla [104].

Although adrenal PCCs are mostly benign, they cause excessive catecholamine production and release, potentially leading to a hypertensive crisis. Remarkably, approximately 40% of PPGLs develop due to germline mutations in various genes, making them the most heritable of all human tumours [105]. More than 20 gene mutations associated with PPGL have been identified.

They are categorized into three major clusters with distinct biochemical profile, clinical presentation and prognosis: pseudohypoxia (Cluster 1A and 1B), kinase signalling (Cluster 2), and Wnt signalling (Cluster 3) [106]. Cluster 1 tumours exhibit a pseudohypoxic transcriptional profile and hypermethylation. They include mutations in TCA cycle genes (SDHx, IDH1/2, FH, and MDH2), as well as mutations in VHL, EPAS1, and EGLN1/2. Cluster 2 tumours are characterized by enriched kinase-signalling transcripts and a lower degree of DNA methylation. Key mutations include NF1, RET, and HRAS [107]. Cluster 3 tumours have increased expression of Wnt pathway genes, including CSDE1 mutations and MAML3 fusions. These alterations drive angiogenesis, cell proliferation, survival, invasion, metastasis, and metabolic deregulation [108]. A schematic of the PPGL clusters is illustrated in Figure 6.

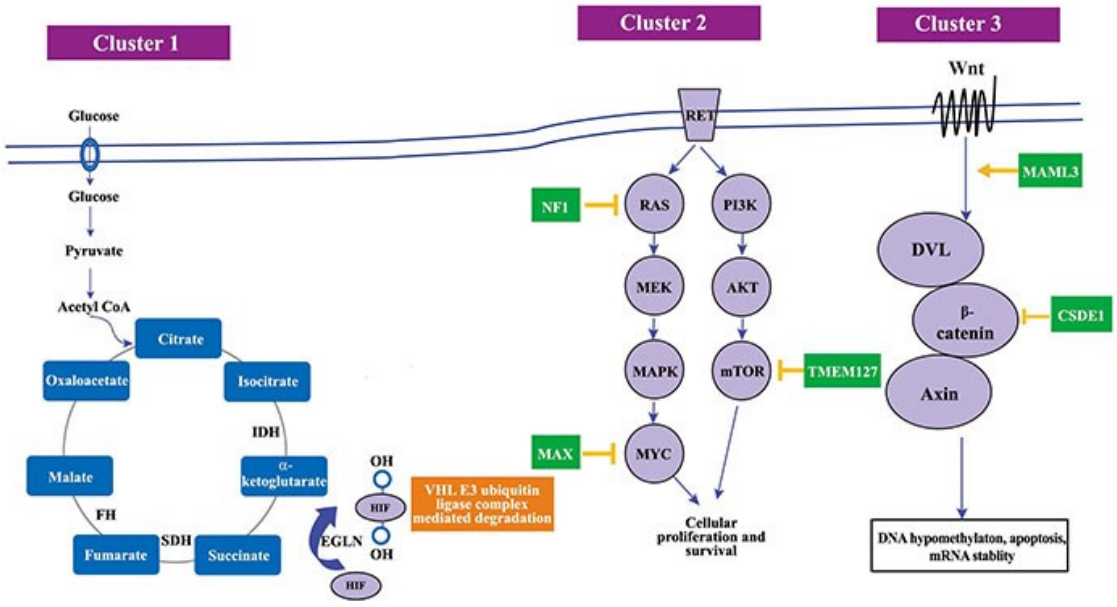

Figure 6: Overview of PPGL clusters. Adapted from [109].

The primary treatment for PPGL is surgical resection. For advanced or metastatic cases, chemotherapy, particularly the CVD regimen (cyclophosphamide, vincristine, and dacarbazine), or temozolomide is a well-established approach, especially for patients with SDHB mutations [106,110]. Additionally, tyrosine kinase inhibitors (TKIs) targeting angiogenesis have shown efficacy, particularly in metastatic SDHB-mutant tumours [111]. However, their use is often associated with significant cumulative side effects, and the development of tumour resistance [112].

Mutations in SDHB are the most common among CII subunits within Cluster 1 tumours. This is due to the inherent susceptibility of the SDHB subunit, where random missense mutations are more likely to disrupt its function. The vulnerability of SDHB is due to the dense network of amino acid interactions with other subunits and essential components of CII, such as FAD, heme, ubiquinone, and [Fe-S] clusters [113].

Other possible explanations include the idea that SDH subcomplexes remaining after the loss of SDHB may acquire tumourigenic properties, such as the presence of CII_{low}, an alternative subassembly of CII with a molecular weight lower than that of SDHA [114]. Additionally, SDHB may possess other, yet undiscovered, tumour-suppressor functions. Studies in yeast models of SDHB deficiency have demonstrated that residual SDHA can persist and may negatively impact cellular function [115]. Moreover, because of reduced catecholamine production in PPGL with pathogenic SDHB variants, these tumours grow without symptoms for a long time [116]. Additionally, PPGL patients with SDHB variants tend to present with a more aggressive clinical phenotype, including a higher risk of metastasis. These tumours have some of the highest rates of disease-specific morbidity and mortality among all hereditary PPGL types [117].

Recent studies suggest that the loss of CII alone does not trigger tumour formation. SDHB-deficient chromaffin cells accumulate succinate, experience mitochondrial swelling, and histone hypermethylation. However, this does not result in increased proliferation, HIF1/2α accumulation, or significant changes in catecholamine levels. Instead, mice with SDHB-deficient chromaffin cells develop obesity [118]. A similar non-diabetic obese phenotype was observed in SDHC-deficient mice, linked to hypothalamic neuron loss [94].

In the mouse adrenal medulla, loss of SDHB is compatible with cell survival, likely due to the high levels of ascorbate in the adrenal gland. Interestingly, an additional genetic alteration may be required for tumourigenesis in SDHB-deficient cells. For instance, loss of NF1, commonly deregulated in Cluster 2 PPGL, can induce tumours resembling those in Cluster 1 PPGL (SDHB-deficient) [119]. Furthermore, a recent study demonstrated that conditional knockout of SDHC in neuroendocrine cells caused developmental defects but did not result in tumour

formation [120]. These findings suggest that the loss of another gene, likely located near the SDH gene on the 1p arm, may be necessary for tumour formation [118].

1.3.2.2. Renal cell carcinoma

Each year, more than 400,000 patients are diagnosed with renal cancer, making it one of the most common cancers worldwide [121]. Patients with localized RCC have a favourable 5-year survival rate of 73%. However, up to 30% of patients develop metastatic disease [122]. Despite advances in targeted and immune therapies, patients with metastatic RCC continue to have poor outcomes, with a median progression-free survival of just 15 months [123].

The WHO classification defines over 14 types of renal cell carcinomas (RCC) [124], with clear cell (ccRCC), papillary (pRCC), and chromophobe RCC (chRCC) being the most common. RCC grading has been an essential clinical tool for nearly a century. The Fuhrman grading system was the first to establish a correlation between nuclear size, cell shape, and patient prognosis [125]. In 2016, the Fuhrman system was replaced by the WHO/International Society of Urological Pathology (WHO/ISUP) grading system, which classifies RCC from Grade 1 to Grade 4 across all subtypes. The WHO/ISUP grade is primarily based on nucleolar features, with Grade 4 including additional characteristics such as nuclear pleomorphism, and rhabdoid or sarcomatoid differentiation. Grades 3–4 are associated with approximately a twofold increase in the risk of metastatic RCC [126] and are strong predictors of recurrence, indicating their impact on overall survival [127]. However, pRCC and chRCC show lower variation in nuclear features and architectural complexity compared to ccRCC, thus, grading is less predictive of prognosis [128].

1.3.2.2.1. Clear cell renal carcinoma (ccRCC)

ccRCC accounts for approximately 75% of all RCC cases, making it the most common subtype [129]. About 95% of ccRCC cases are sporadic, while the remaining 5% are associated with the hereditary Von Hippel-Lindau (VHL) syndrome. **VHL syndrome** is caused by germline mutations in the VHL gene, predisposing individuals to ccRCC, hemangioblastomas, pancreatic neuroendocrine tumours, and pheochromocytoma/paraganglioma (PCC/PPGL). The syndrome is classified into two main subtypes based on the mutation type and clinical presentation [130].

Type 1 VHL disease, typically resulting from truncating or missense VHL mutations, is characterized by a high risk of ccRCC and HB but a low risk of developing PPGL. In contrast, type 2 VHL disease results from VHL missense mutations and is associated with PPGL. Interestingly, individuals from the Chuvash region who are homozygous for the VHL R200W mutation, present with congenital erythrocytosis. This condition, known as Chuvash polycythemia, is characterized by excessive erythrocyte production due to HIF pathway defects, which lead to increased production of erythropoietin [131].

The VHL protein (pVHL) plays a central role in oxygen sensing by regulating the stability of HIFs. In an environment with normal oxygen levels, pVHL functions as part of the E3 ubiquitin ligase complex, recognizing and binding to proline residues in the oxygen-dependent degradation domains of hydroxylated HIF-α subunits. This interaction targets HIF-α for ubiquitination and subsequent degradation by the proteasome. Hydroxylation of HIF-α by prolyl hydroxylase domain (PHD) proteins is oxygen-dependent, linking the degradation of HIFs to oxygen availability [132].

In hypoxic conditions or the absence of functional pVHL, HIF-α subunits escape degradation, accumulate, and activate transcription of their target genes. HIFs are heterodimeric transcription factors, consisting of an oxygen-sensitive HIF-α subunit and a stable HIF-1β subunit. Among them, HIF-1 (HIF-1α/HIF-1β) and HIF-2 (HIF-2α/HIF-1β) are particularly significant in the development of clear cell renal carcinoma (ccRCC), as they regulate the expression of thousands of genes [133]. These transcription factors share overlapping functions but also regulate distinct sets of genes [134]. Notably, recent research suggests that HIF-1α may act as a tumour suppressor, while HIF-2α predominantly promotes ccRCC progression [135].

When HIF-2α accumulates, it dimerizes with HIF-1β, translocates to the nucleus, and binds hypoxia response elements in gene promoters, activating transcription of genes such as VEGF, PDGF, and carbonic anhydrase IX (CAIX). CAIX is essential for hypoxic adaptation, regulating pH by converting carbon dioxide into bicarbonate and protons[136]. Transcription of these genes enhances tumour angiogenesis, proliferation, and metastasis [137]. Beyond angiogenesis, HIFs also drive glycolytic rate by upregulating genes encoding glycolytic

enzymes, further supporting tumour growth under low-oxygen conditions [138]. A schematic of the VHL-HIF pathway is illustrated in Figure 7.

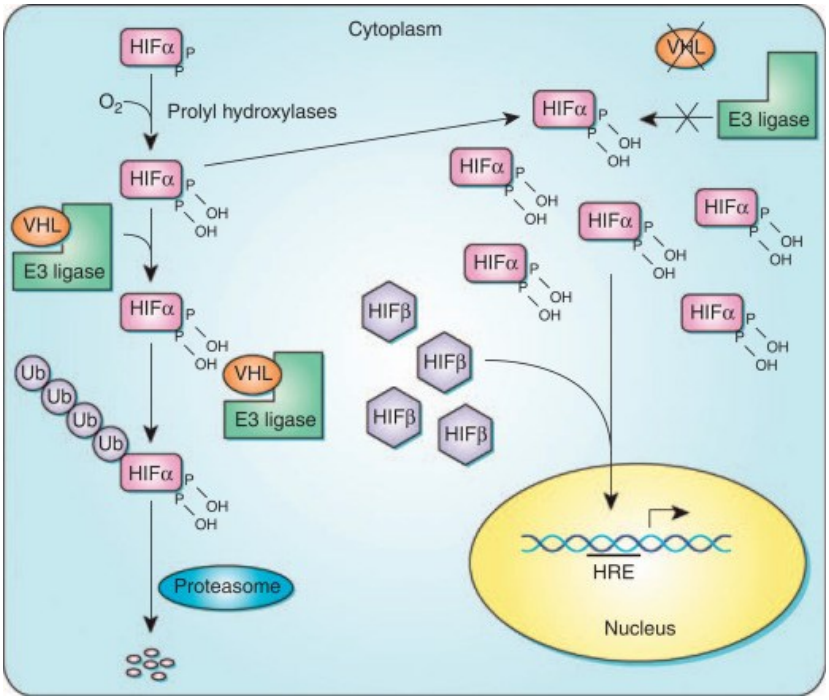

Figure 7: Overview of the VHL-HIF pathway. Adapted from [132].

The VHL gene is universally expressed, but mutations in this tumour suppressor gene are associated with a limited range of tumour types. Interestingly, VHL is categorized as a "common essential gene", meaning that its inactivation in most cancer cell types leads to a significant loss of cellular fitness [139]. However, ccRCC stands out as an exception. This cancer type, which frequently harbors VHL mutations, represents the primary cause of mortality in the VHL syndrome, with affected individuals facing a 50–70% lifetime risk of developing ccRCC. Moreover, sporadic ccRCC also heavily relies on VHL loss, as mutations or inactivation of this gene are found in 90–95% of such tumours [140].

Among VHL disease cases that manifest as PPGL, most carry missense VHL mutations. Conversely, patients with null VHL alleles typically develop RCC and HBs without increased risk of PPGL. This is unexpected given that pVHL suppresses the transcription factor HIF2, whose gain-of-function mutations are also linked to PPGL. Complete loss of pVHL disrupts

neuroblastoma cell line fitness in a HIF2-dependent manner. However, this fitness impairment can be rescued by pVHL variants associated with PPGL, but not by variants tied to a lower risk of the disease. These findings indicate that excessive HIF2 activity beyond a specific threshold prevents PPGL development [141].

Studies have shown that the most frequent genomic alterations in ccRCC involve the loss of chromosome 3p, as seen in 91% of ccRCC samples. This deletion includes the loss of the VHL gene and nearby tumour suppressor genes, such as PBRM1, BAP1, and SETD2, all of which are chromatin modifiers [142]. These genes become targets for inactivating mutations in the second copy of the chromosome. The accumulation of driver mutations correlates with tumour stage, grade, and necrosis [143,144]. While over 90% of ccRCC cases exhibit second-hit mutations in VHL, complete loss of VHL expression alone is not enough to initiate ccRCC in humans or mice [145,146].

Treatment and management of ccRCC depend on the disease stage. For localized tumours, complete surgical resection through nephron-sparing partial nephrectomy or radical nephrectomy is recommended. First-line treatment for metastatic ccRCC includes tyrosine kinase inhibitors targeting VEGF, such as sunitinib, sorafenib, pazopanib, and axitinib, as well as rapamycin inhibitors like temsirolimus and everolimus [147]. Given the reliance of ccRCC on HIF activation, particularly HIF2, HIF2 inhibitors like belzutifan have been developed [148]. The prognosis for advanced RCC remains poor due to its low response to radiotherapy and chemotherapy and resistance to treatment [149].

1.3.2.2.2. Papillary RCC

The second most common type of RCC is pRCC, representing approximately 15% of all cases. pRCC is further divided into two histologic subtypes: type I and type II. Type I is more common, grows slowly with a 5-year cancer-specific survival of 95%, and includes tumours with MET mutations and chromosome-7 amplification [150]. Type II is more aggressive, with a 5-year cancer-specific survival of 76%, and includes tumours with TCA enzyme mutations, such as that of FH, leading to a highly aerobic glycolytic phenotype and upregulation of the

Nrf2-antioxidant response pathway [151].

1.3.2.2.3. Chromophobe RCC

Chromophobe RCC (chRCC) is the third most common type of RCC, accounting for about 5% of cases. It is a slow-growing tumour that originates in the distal nephron, with a 5-year survival rate of 93% [152]. Most chRCC cases exhibit loss of heterozygosity in chromosomes 1, 2, 6, 10, 13, 17, and 21 [153]. The somatic mutation rate in chRCC is relatively low compared to ccRCC and pRCC and approximately 40% of cases do not present any identifiable driver mutations. Among the few genes consistently mutated across chRCC cohorts, TP53 is altered in 32%, and PTEN in 9% of cases [154].

1.3.2.2.4. SDH-deficient RCC

In addition to PPGL and GIST, there is growing evidence that patients with germline mutations in SDH subunit genes are also at risk of developing RCC, accounting for 0.05-0.5% of all RCC cases [155]. This subtype was introduced in the 2016 WHO classification of renal tumours [124]. These tumours are most commonly characterized by mutations in SDHB, with fewer cases involving mutations in SDHC and SDHA [156]. Similar to PPGL and GIST in these patients, SDH-deficient RCC tumours often lack immunohistochemical labeling for SDHB in the neoplastic cells [155].

To sustain growth, cells deficient in CII need to reprogram their metabolism. In normal cells, NADH and $FADH_2$ produced in the TCA cycle are utilized to synthesize aspartate, which is essential for the production of purines and pyrimidines necessary for cell proliferation. Wild-type cells typically obtain aspartate from glutamine via the oxidative TCA cycle [9]. However, in SDH-deficient cells, the TCA cycle is dysfunctional, requiring alternative metabolic pathways. Specifically, these cells rely on pyruvate carboxylation and a reductive TCA cycle.

Pyruvate carboxylation in SDH-deficient cells involves the use of pyruvate carboxylase instead of PDH to convert glucose into acetyl-CoA via glycolysis. This enzyme, found to be

upregulated in SDHB KO cells, produces OAA, which is then converted into aspartate through glutamate-oxaloacetate transaminase 2 (GOT2) [157,158]. The pyruvate carboxylation pathway is illustrated in Figure 8.

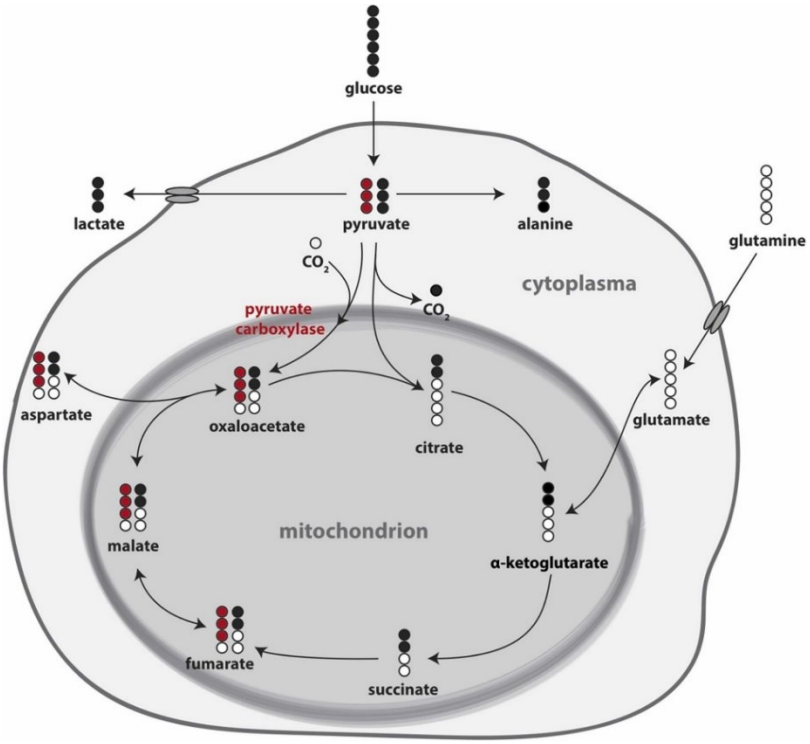

Figure 8: Pyruvate carboxylation pathway in cancer. Adapted from [159].

In the reductive TCA cycle (see Chapter 1.1.3.), glutamine plays a major role in biomass synthesis via reductive carboxylation [160–162]. Reversible transamination between glutamate and pyruvate generates αKG and alanine. αKG can be reduced through reductive carboxylation to form citrate [163,164]. Citrate is then converted to OAA and aspartate by glutamate-oxaloacetate transaminase 1 (GOT1) [160].

2. Research aims

This dissertation investigates the role of CII in cancer, drawing on patient-derived samples and cell line models. Through detailed analysis of CII status and assembly, this research aims to explore the link between mitochondrial alterations and tumour progression in the context of CII.

Aim 1: Investigate mitochondrial content and metabolic profiles in RCC subtypes

- Assess mtDNA levels, protein content, and metabolic activity in different subtypes of RCC, focusing on ccRCC, pRCC, and chRCC.
- Determine the relationship between mitochondrial content and the metabolic adaptations unique to ccRCC, especially the shift towards glycolysis observed in ccRCC.

Aim 2: Characterize CII function across RCC subtypes

- Evaluate the functional status of CII in RCC tumours, with a specific focus on its assembly, and activity.
- Correlate these findings with clinical characteristics, including tumour grade, subtype, and VHL status, to identify CII-specific metabolic vulnerabilities.

Aim 3: Explore the role of CII assembly factors in SDHA maturation

- Investigate the role of CII assembly factors, SDHAF2 and SDHAF4 in the maturation of SDHA in a PPGL cell line.
- Explore how disruptions in assembly processes contribute to metabolic changes, succinate accumulation, and the formation of CII subassemblies, linking these mechanisms to cancer progression and therapeutic opportunities.

3. List of methods

Outlined below are the methods used throughout the research conducted for this thesis, with further details provided in the referenced publications. Each method is associated with the relevant research articles, denoted as research article 1 (see Chapter 4.1) and/or research article 2 (see Chapter 4.2).

Patient tissue specimen handling (1)

Cell culture

- Primary cell culture (1)
- Patient-derived organoid formation (1)
- hPheo1 cell line (2)

Histology and immunohistochemistry (1)

Immunofluorescence (1)

Genetic manipulation and cloning techniques

- Generation of hPheo1 SDHAF2 and/or SDHAF4 KO and reconstituted cells using the CRISPR/Cpf1 gene editing technology (2)

Isolation of mitochondria (1, 2)

Electrophoretic methods and western blotting

- SDS-PAGE (1, 2)
- BN-PAGE (1, 2)

In-gel SDH activity (1, 2)

Succinate-quinone reductase (SQR) activity (1, 2)

Gas chromatography-mass spectrometry (GC-MS)

- Succinate-to-fumarate ratio determination (2)

Isolation of nucleic acids

- DNA isolation (1)
- RNA isolation (1, 2)

Quantitative polymerase chain reaction (qPCR)

- Determination of mtDNA level (qPCR) (1)
- Gene expression level determination (qRT-PCR) (1, 2)

Next-generation sequencing of the VHL gene (1)

High-resolution respirometry (1, 2)

Citrate synthase activity (1)

Statistical analysis (1, 2)

4. Results

This dissertation thesis is based on two original research articles, one first-author and one co-author publication. The relevance of these publications in the dissertation as well as the contribution of the author is described below.

4.1. **Miklovicova, S.,** Volpini, L., Sanovec, O., Monaco, F., Hadrava Vanova, K., Novak, J., Boukalova, S., Zobalova, R., Klezl, P., Tomasetti, M., Bobek, V., Fiala, V., Vcelak, J., Santarelli, L., Bielcikova, Z., Komrskova, K., Kolostova, K., Pacak, K., Dvorakova, S., Neuzil, J., 2025. Mitochondrial respiratory complex II is altered in renal carcinoma. *Biochimica Biophysica Acta – Molecular Basis of Disease*, 1871(1):167556. https://doi.org/10.1016/j.bbadis.2024.167556

CII plays a dual role in mitochondrial energy metabolism and redox balance, making its function and assembly critical to cellular homeostasis. In RCC, metabolic reprogramming, including alterations in the TCA, is a hallmark feature. Investigating CII function and assembly in these tumours is vital, as its dysregulation could drive tumour progression.

We analyzed both tumour and adjacent healthy kidney tissues from 78 RCC patients undergoing nephrectomy, encompassing the three most common histotypes, namely ccRCC, the predominant RCC subtype, followed by pRCC and chRCC. Patients were stratified into low- and high-grade groups according to the WHO/ISUP grading system, correlating with prognostic outcomes. Our evaluation included the identification of VHL mutations and CAIX expression in ccRCC. We assessed mitochondrial CII respiration, CIV activity and CS activity in fresh tissues. Utilizing SDS-PAGE/WB, we identified selected renal carcinoma-associated proteins and CII subunits. We analyzed gene expression of key CII genes and mtDNA content. Furthermore, NBGE/WB on isolated mitochondria revealed details on CII assembly, complemented by in-gel assays for CII activity. Additionally, we characterized organoids derived from tumour tissue of 7 patients.

Our results revealed high mitochondria content and CII activity in normal kidney tissue of RCC patients, indicating high energy demands of this tissue. Interestingly, all assessed mitochondrial

parameters in pRCC and chRCC tissues were comparable to normal kidney tissue. On the contrary, we observed a marked reduction in these parameters, namely mtDNA, mitochondrial protein content and function, alongside diminished CII activity in ccRCC tissues compared to normal kidney tissue. These tumours, characterized by variants in the VHL gene and CAIX expression, exhibited increased mitochondrial content in high-grade cases. However, CII function remained compromised in these tumours, indicating metabolic changes linked to tumour aggressiveness. Disrupted CII assembly in ccRCC was accompanied by the emergence of a CII_{low} subassembly, recently described by our group to consist of the catalytic subunit SDHA and potentially one or two CII assembly factors (see Chapter 4.2.) Tumor organoids derived from ccRCC closely resembled the original tumor tissue in terms of structure, genome, and molecular characteristics.

These findings demonstrate that the integrity of complex II, as indicated by its assembly and SDHB levels, diminishes with the advancing severity of ccRCC. Such insights could be important for patient stratification and the development of targeted therapeutic strategies.

Contribution to the publication: I participated in the study design and contributed to the majority of the experimental work, data analysis and writing of the original draft as well as revision.

Beyond the scope of this article, we found that decreased mitochondrial content and OXPHOS utilization in ccRCC were accompanied by increased glycolytic capacity, based on the expression of key glycolytic enzymes and metabolic regulators. Notably, we found elevated levels of hexokinase-2 (HK-2), phosphofructokinase (PFKP) and pyruvate kinase M2 (PKM2) in ccRCC compared to healthy kidney tissue, while the levels of pyruvate dehydrogenase (PDH) were decreased, which would otherwise feed acetyl-CoA into the TCA cycle, contributing to our findings of suppressed TCA cycle enzyme, CS. The results, shown in Figure 9, highlight the addiction of ccRCC to glycolysis in energy generation and macromolecule synthesis.

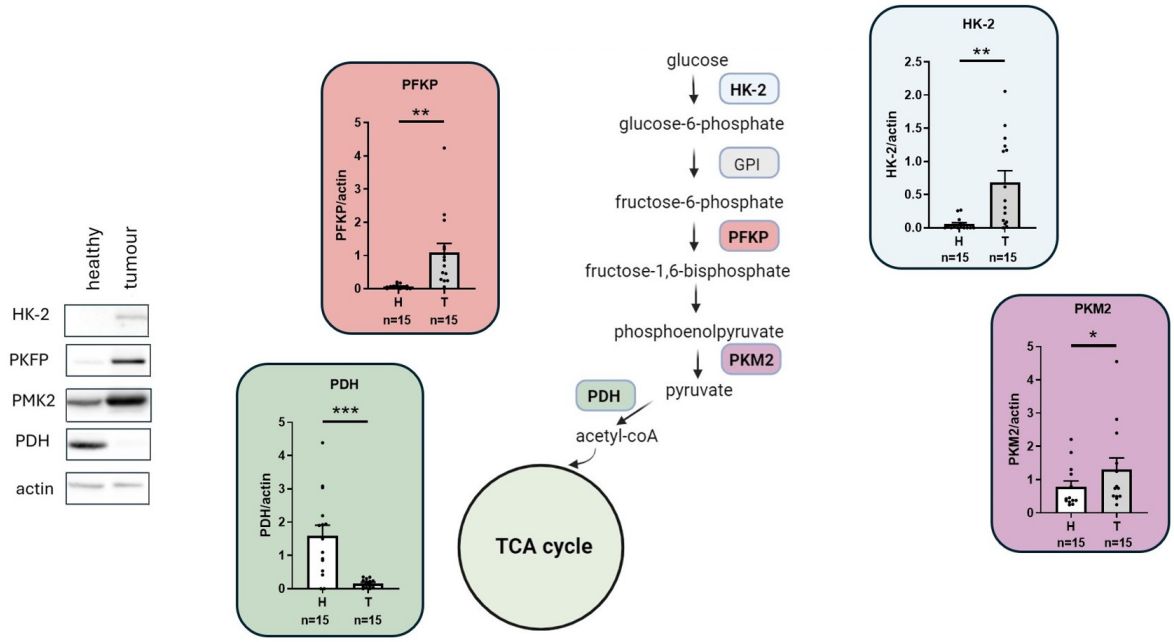

Figure 9: Expression of selected glycolytic enzymes and metabolic regulators in ccRCC tumours. H: healthy kidney; T: tumour.

4.2. Sharma, P., Maklashina, E., Voehler, M., **Balintova, S.,** Dvorakova, S., Kraus, M., Hadrava Vanova, K., Nahacka, Z., Zobalova, R., Boukalova, S., Cunatova, K., Mracek, T., Ghayee, H. K., Pacak, K., Rohlena, J., Neuzil, J., Cecchini, G., Iverson, T. M., 2024. Disordered-to-ordered transitions in assembly factors allow the complex II catalytic subunit to switch binding partners. *Nature Communications*, 15:473. https://doi.org/10.1038/s41467-023-44563-7

Our findings regarding the identification of CII_{low} in high-grade renal tumours (see Chapter 4.1.) were complemented by our detailed investigation of this assembly species. The assembly of CII depends on precise interactions between the catalytic subunit SDHA, FAD, and the assembly factors SDHAF2 and SDHAF4. Despite its importance, the sequence of these interactions, the specific roles of SDHAF2 and SDHAF4 in SDHA maturation, and the structural features of intermediate complexes remain poorly understood.

To explore this, we utilized the human adrenal pheochromocytoma cell line hPheo1. We

generated two SDHAF4 KO clones, an SDHAF2 KO clone, and an SDHAF2/SDHAF4 double KO clone, and re-expressed SDHAF4 in all these lines. These cell lines were characterized by assessing protein levels, respiration, SDH activity, SQR activity, and the succinate-to-fumarate ratio.

Our results show that the loss of SDHAF4 did not affect SDHA levels but led to decreased SDHB levels and an increase in SDHAF2, which highlights the critical role of SDHAF4 in SDHA maturation. SDHAF4 KO resulted in impaired CII function, as reflected by reduced CII-dependent respiration, and SDH and SQR activity, accompanied by an elevated succinate-to-fumarate ratio. Re-expression of SDHAF4 restored these parameters to levels comparable to parental cells.

Using blue native- (BN-)PAGE, we demonstrated that in parental cells, SDHA assembled into mature CII as expected. However, in SDHAF4 KO cells, SDHA predominantly formed a ~100 kDa complex with SDHAF2, and re-expressing SDHAF4 restored proper CII assembly. Immunoprecipitation revealed strong SDHAF4-SDHA interactions, but only in the presence of SDHAF2, suggesting that SDHAF2 binding to SDHA precedes SDHAF4 association. SDHAF2 KO in SDHAF4 KO cells resulted in SDHA at ~100 kDa, without forming an SDHA-SDHAF4 complex. We also captured the SDHA-SDHAF2-SDHAF4 intermediate, but only under specific *in vitro* conditions, indicating a rapid transition between these species.

The crystal structures highlighted the importance of intrinsically disordered regions in SDHAF2 and SDHAF4, which facilitate the transition from SDHA-SDHAF2 to SDHA-SDHAF4. These regions in SDHAF4 were particularly critical for binding to the SDHA-SDHAF2 complex and for subsequent SDHAF2 displacement.

Based on these findings, we propose a stepwise model for SDHA maturation: SDHAF2 first binds to SDHA, followed by SDHAF4 binding. The intrinsically disordered regions of these assembly factors play essential roles in this process, providing structural and functional insights into the dynamics of CII assembly and its regulation.

Contribution to the publication: I contributed to this publication with experimental work related to the characterization of cell lines. I also participated in manuscript writing and revision.

5. Discussion

Among the most energy-demanding organs in the body, the kidney has a metabolic rate that exceeds many others at rest [165]. It has very high mitochondrial density and oxygen consumption, which are crucial for its various cellular functions, including waste excretion, nutrient reabsorption, fluid homeostasis, and blood pressure regulation. Most of the filtrate from the glomerulus is reabsorbed by the proximal tubules, which contain more mitochondria than any other part of the kidney [166]. Interestingly, nephron progenitors, self-renewing cells that form nephrons, rely on glycolysis for energy in early development but transition to OXPHOS as they differentiate. VHL, a key regulator of this metabolic shift, is essential for progenitor differentiation and proper kidney development [167].

There is conflicting evidence regarding the metabolic changes that occur in kidney cells when they become cancerous. Some studies suggest global metabolic reprogramming in kidney tumours [168–170]. Others argue that kidney tumours retain the metabolic characteristics of their tissue of origin, as they derive nutrients from the same source, although this particular study was unselected with regard to tumour histology [171].

There are three main histological subtypes of kidney cancer: ccRCC, pRCC, and chRCC. A distinct subtype of RCC, characterized by germline mutations in CII, has also been identified, representing about 0.5% of all kidney cancers [124]. However, other evidence suggests that CII dysfunction per se may be more widespread in kidney tumours [172]. With these considerations in mind, the primary goal of this project was to explore mitochondrial characteristics and the CII status across different subtypes of RCC.

We found that pRCC and chRCC exhibit mitochondrial content and function similar to that of adjacent healthy kidney tissue. In contrast to ccRCC, the metabolic characteristics of pRCC and chRCC remain less well understood, largely due to their lower prevalence. Our study contributes to a better understanding by showing that both pRCC and chRCC retain mitochondrial content and function, including CII activity. These findings contradict previous reports suggesting a reduction in ETC activity in chRCC [173,174], emphasizing the

importance of deeper investigation into the metabolic diversity across different RCC subtypes.

Our results are consistent with prior studies showing that while chromophobe RCC may harbor mutations in CI, other ETC complexes (CIII, CIV, and CV) are upregulated in these tumours. Similarly, higher levels of mitochondrial RNA (mtRNA) have been observed in chRCC and oncocytomas compared to normal kidney tissue [175]. However, this study proposed that somatic mutations in mtDNA that impair OXPHOS lead to dysfunctional mitochondria that are not cleared due to defective mitophagy. Under this scenario, chRCC cells are thought to enter an energy crisis, compensated by upregulating the biogenesis of dysfunctional mitochondria. Thus, elevated mtRNA levels in chRCC tumours may be expected to reflect a decline in mitochondrial respiration [176]. Importantly, our respirometry data show mitochondrial respiration levels comparable to normal kidney tissue. This discrepancy suggests that the previously proposed model may need further exploration to fully understand the metabolic adaptations in RCC subtypes.

In contrast to chRCC and pRCC, we found decreased mitochondria content and activity specifically in ccRCC tumours compared to healthy kidney tissue. These alterations in mitochondrial function may result from suppressed mtDNA content, as we claim by a lower mtDNA/nDNA ratio in ccRCC, which could be associated with reduced expression of mtDNA-encoded genes. Previous studies have shown that decreased mtDNA content can lead to downregulation of ETC complex subunits, suggesting a direct connection between reduction in mtDNA and downregulation of ETC proteins [174]. Thus, low mtDNA content may contribute to impaired OXPHOS in ccRCC.

In ccRCC, reduced expression of mitochondrial transcription factor A (TFAM) leads to impaired replication and transcription of mtDNA [177]. As a result, reduced TFAM-mediated regulation of mtDNA likely affects the observed decrease in mtDNA content in ccRCC tumors. Additionally, TFAM expression has been shown to be downregulated in ccRCC due to reduced levels of the co-activator protein PGC1α. [178]. Moreover, wild-type VHL plays a role in protecting TFAM from degradation by the LON protease in kidney tissue. However, when VHL is mutated, TFAM is no longer protected, leading to its degradation and impaired mitochondrial

biogenesis in ccRCC tumors [179]. This suggests that mitochondrial metabolism is overall suppressed in ccRCC tumors compared to adjacent healthy kidney tissue, which may account for the differences observed between ccRCC and other RCC subtypes.

Although mitochondrial dysfunction is evident in ccRCC, the cells can compensate by relying on glycolysis and alternative metabolic pathways to maintain energy production and support biosynthetic processes, thereby promoting tumor growth [169]. Our analysis indicates that glycolysis is extensively utilized in ccRCC tumours, as reflected by the upregulation of key glycolytic enzymes and a reduction in PDH expression compared to adjacent healthy kidney tissue. Since our analysis focused solely on ccRCC tumours, we can only speculate about glycolytic capacities in other subtypes of RCC. Interestingly, previous studies using immunohistochemistry and transcriptomic profiling have shown elevated expression of G6PD, GLS, ACSS2, and ATP5B in pRCC compared to ccRCC, all of which are involved, directly or indirectly (in the case of G6PD), in OXPHOS and ETC. In contrast, genes encoding the key glycolytic enzymes, such as SLC2A1 (which encodes GLUT1), HK-2, and PFKP, were significantly lower in pRCC compared to ccRCC, suggesting reduced glycolytic capacity in this RCC subtype. Furthermore, pRCC tumours did not exhibit increased GLUT1 expression, and glycolytic enzymes such as HK-2 and PFKP showed low expression. On the other hand, increased ATP5B expression in pRCCs, coupled with higher expression of COX IV and TOM20, points to enhanced mitochondrial oxidative capacity in this subtype [180].

Additionally, we observe decreased CII-dependent respiration in ccRCC tissue, which is indicative of compromised CII function. Furthermore, compared to nearby healthy kidney tissue, ccRCC showed a marked decrease in SQR activity. High expression of NDUFA4L2, a HIF-targeted protein that inhibits OXPHOS by interfering with both CI- and CII-dependent respiration in skeletal muscle and, more crucially, in ccRCC, may be linked to suppressed CII activity in ccRCC tumors [181–183]. In light of these results, NDUFA4L2 has been associated with poor survival outcomes and may be a useful marker for ccRCC [182]. Our findings provide additional evidence to the growing theory that ccRCC is a unique subtype of RCC characterized by dysregulated mitochondrial activity, particularly in relation to CII activity. Notably, CII

dysfunction was exclusive to ccRCC and was not observed in pRCC, chRCC, or oncocytoma, suggesting that these RCC subtypes exhibit different metabolic profiles.

In several cancers, reduced mtDNA copy number, lower mtRNA levels, and decreased expression of nuclear-encoded respiratory proteins indicate a decline in mitochondrial respiratory activity [175]. In our study, we found a decrease in mitochondrial content in ccRCC, likely linked to reduced mtDNA copy number (e.g., mt-CO1 levels, as discussed above). This decrease in mitochondrial content was accompanied by a reduction in the expression of nuclear-encoded OXPHOS genes, such as those coding for CII subunits. These findings align with previous studies reporting consistent differential expression patterns between mitochondria and nuclear-encoded OXPHOS subunits [175]. The interdependence of ETC complexes also plays a role as disruption of any single complex can lead to the downregulation of other complexes. More specifically, suppression of CI level in such cases has been linked to decreased mitochondrial ribosome function and impaired mitochondrial protein translation, even though CII is encoded by nuclear DNA [184].

Within the context of SDH-deficient RCC, previous studies showed that tumours with SDHC and SDHD mutations primarily resemble ccRCC microscopically. In contrast, SDHB-mutated RCCs, which are the most common, show more diverse histological features that overlap with several RCC subtypes, including chRCC, ccRCC, pRCC, sarcomatoid RCC, unclassified RCC, and renal oncocytoma [185]. Notably, SDHB-mutated RCCs are negative for CAIX, which distinguishes them from ccRCC and suggests that the effects of SDHB mutations may not be dependent on the VHL/HIF/CAIX axis, as is often seen in ccRCC [185]. Ultrastructural studies of SDHB-deficient RCCs showed abundant mitochondria in the cytoplasm, with cytoplasmic inclusions corresponding to abnormal mitochondria exhibiting degenerated cristae and matrix [186,187]. Importantly, these SDHB-deficient tumours retain SDHA immunostaining [185]. Based on our findings, we propose that SDH dysfunction in germline SDH-mutant RCC, representing only 0.5% of all kidney cancers [172,188], is distinct from the mitochondrial dysfunction seen in ccRCC, with the underlying molecular mechanisms differing. In ccRCC, CII downregulation is likely VHL/HIF/CAIX axis-dependent, whereas in SDH-mutant RCC, it is rather genetically determined by mutations in the SDH complex. This distinction highlights how

metabolic alterations in RCC can be driven by different genetic mechanisms: either via adaptive responses to hypoxia in ccRCC or by means of inherited mutations in CII in SDH-deficient tumours.

Findings from this work and by others are in line with recent studies that identify CII dysfunction asa negative prognostic factor in RCC, with a distinct subset of SDH-deficient tumours harbouring mutations in CII [168,189–191]. The reduced CII activity in ccRCC is particularly noteworthy, as CII subunits, unlike other ETC complexes, CII subunits are exclusively encoded by nuclear DNA.

Transcriptional regulation of ETC proteins involves both nuclear- and mitochondrial-encoded genes, which are coordinately controlled by several key nuclear factors (e.g., NRF-1, NRF-2, and ERRα) and co-activators from the PGC-1 family (e.g., PGC-1α and PRC). When PGC-1 activity is reduced, it acts as a coactivator or direct activator of several transcription factors, including NRF-1 and NRF-2, which are key regulators of mitochondrial function. In aerobic cardiomyocytes, NRF-1 has been shown to regulate SDH expression, with suppression of NRF-1 specifically leading to decreased SDHA subunit expression. This mechanism could explain the observed link between reduced mitochondrial content and decreased CII activity in ccRCC tumours [192].

Other studies have suggested that the downregulation of CII in ccRCC may involve SDHB promoter methylation [193,194], deletions of SDHB and inhibition of SDHD by miRNAs [172]. Notably, SDHD inhibition is regulated by HIF–dependent upregulation of miR-210, which is induced by loss of VHL and directly inhibits the SDHD transcript. Interestingly, the extent of VHL loss correlates with the level of miR-210 upregulation and subsequent SDHD loss. This aligns with the well-established notion that a single functional allele of VHL is sufficient to prevent RCC carcinogenesis and that a second mutation in the preserved allele is necessary to initiate tumourigenesis [172]. These findings suggest that low CII levels in ccRCC are not simply a consequence of reduced mitochondrial content, but rather reflect a distinct pathological phenomenon driven by specific mechanisms. This mechanism differs from the CII dysfunction observed in ccRCC tumours with germline mutations in CII.

After observing a suppression of mitochondrial metabolism and CII content in ccRCC, we aimed to investigate their potential impact on disease progression. Tumor histological grade is a key prognostic factor in cancer, with higher-grade tumors frequently associated with greater metastatic potential and more aggressive disease. High-grade RCC tumours also tend to exhibit immunosuppressive traits, which are generally unfavourable for patient prognosis [195].

To investigate the relationship between metabolic changes and tumour grade, we compared high- and low-grade RCC tumours. We found that mitochondrial content and activity increased with tumour grade. Notably, high-grade tumors exhibited higher mtDNA content and CS activity compared to low-grade tumors [196] and high OXPHOS levels were linked to lower survival in metastatic patients [197].

Our findings align with a recent study that demonstrated the reactivation of OXPHOS in metastatic tumours [188]. Importantly, the same results were observed in high-grade tumours, where elevated OXPHOS scores in high-risk, high-grade tumours from the TCGA cohort were predictive of poor patient outcomes. Additionally, another study using the TCGA cohort showed that higher mtDNA content correlated with poor survival, despite most OXPHOS genes being encoded by nuclear DNA. Together, these findings suggest that both mtDNA abundance and the expression of nuclear-encoded OXPHOS genes are elevated in aggressive primary ccRCCs with unfavourable prognosis [175]. This points to mitochondrial adaptations in primary ccRCC tumours that may facilitate their metastatic potential.

Mitochondrial biogenesis, driven by PGC-1α, has been shown to promote cancer cell motility and metastatic dissemination, as observed in breast cancer [198]. Invading cancer cells rely on mitochondrial biogenesis and increased respiration for their migratory properties. Importantly, enhanced mitochondrial respiration did not affect glycolytic rates in circulating tumour cells and did not affect cancer cell proliferation or growth of primary tumours, suggesting that invasive and migratory traits are more closely linked to mitochondrial respiration than to the proliferative capacity of the primary tumour [198].

Significant genetic changes are now widely recognized as essential for the progression of

advanced-stage RCCs [199]. Evidence suggests that loss of the SETD2 gene, which encodes a histone methyltransferase, is critical apart from VHL for tumour progression, invasion and metastasis in RCC [200]. Loss of SETD2 has also been linked to increased OXPHOS via upregulation of PGC-1α expression [201].

To our surprise, the amounts of the CII subunits SDHA and SDHB, the assembly factor SDHAF2, CII-dependent respiration, and SQR activity did not significantly differ between high-grade and low-grade tumors.

Interestingly, our analysis of CII assembly revealed a decrease in SDHB levels and a lower proportion of fully assembled CII in high-grade tumours. Interestingly, positive reactivity for SDHB in areas with low-grade morphology, transitioning to a high-grade sarcomatoid component with the absence of reactivity were previously found in ccRCC, suggesting that SDHB protein loss might have been the result of additional genetic alterations that developed during tumour progression [202]. In contrast, we observed increased levels of SDHA and SDHAF2 within a sub-assembly of CII, which we previously termed $CII_{low.}$ Additionally, SDH activity was decreased in high-grade tumour mitochondria. These results imply that CII assembly may be more defective, potentially due to a shortage of SDHB and the accumulation of CII_{low}, where SDHA likely associates with SDHAF2 and/or SDHAF4 [114]. The persistence of this dysfunctional CII_{low} sub-assembly may indicate its involvement in tumour progression and metastasis via mechanisms that remain unclear.

The exact cause of the concurrent increase in mitochondrial activity and the disruption in CII assembly in high-grade ccRCC tumors has yet to be identified. Our findings suggest that CII_{low} may serve as a negative prognostic marker in ccRCC, possibly facilitating mechanisms that drive tumour progression and metastasis. The functional significance of CII_{low} remains an open question, although our previous work showed that cells lacking CII_{low} failed to form tumours in a mouse model. These cells were proposed to be less efficient at sensing and adapting to energy demands. While cells with and without CII_{low} grew well in glucose-rich media, those lacking CII_{low} showed poor growth in galactose-rich media and exhibited increased sensitivity to cell death, resembling nutrient deprivation conditions. This suggests that CII_{low} may be important

for sustaining cell growth under nutrient-permissive conditions, which could be particularly relevant in high-grade tumours, where CII_{low} may help the cells survive nutrient scarcity and promote metastasis [114].

Furthermore, the poor growth of CII_{low}-deficient cells in galactose-rich media indicates the reliance of cells with CII_{low} on OXPHOS [114]. Our findings suggest that high-grade tumours are more dependent on OXPHOS, and CII_{low} may play a role in helping these cells cope with the increased oxidative stress associated with higher OXPHOS activity. By having CII_{low}, these cells may better withstand the demands of elevated OXPHOS, enabling them to survive and proliferate even under conditions of low oxygen or nutrient competition, as hypoxia is known to be more pronounced in high-grade tumours. A schematic of the main findings of this study is illustrated in Figure 10.

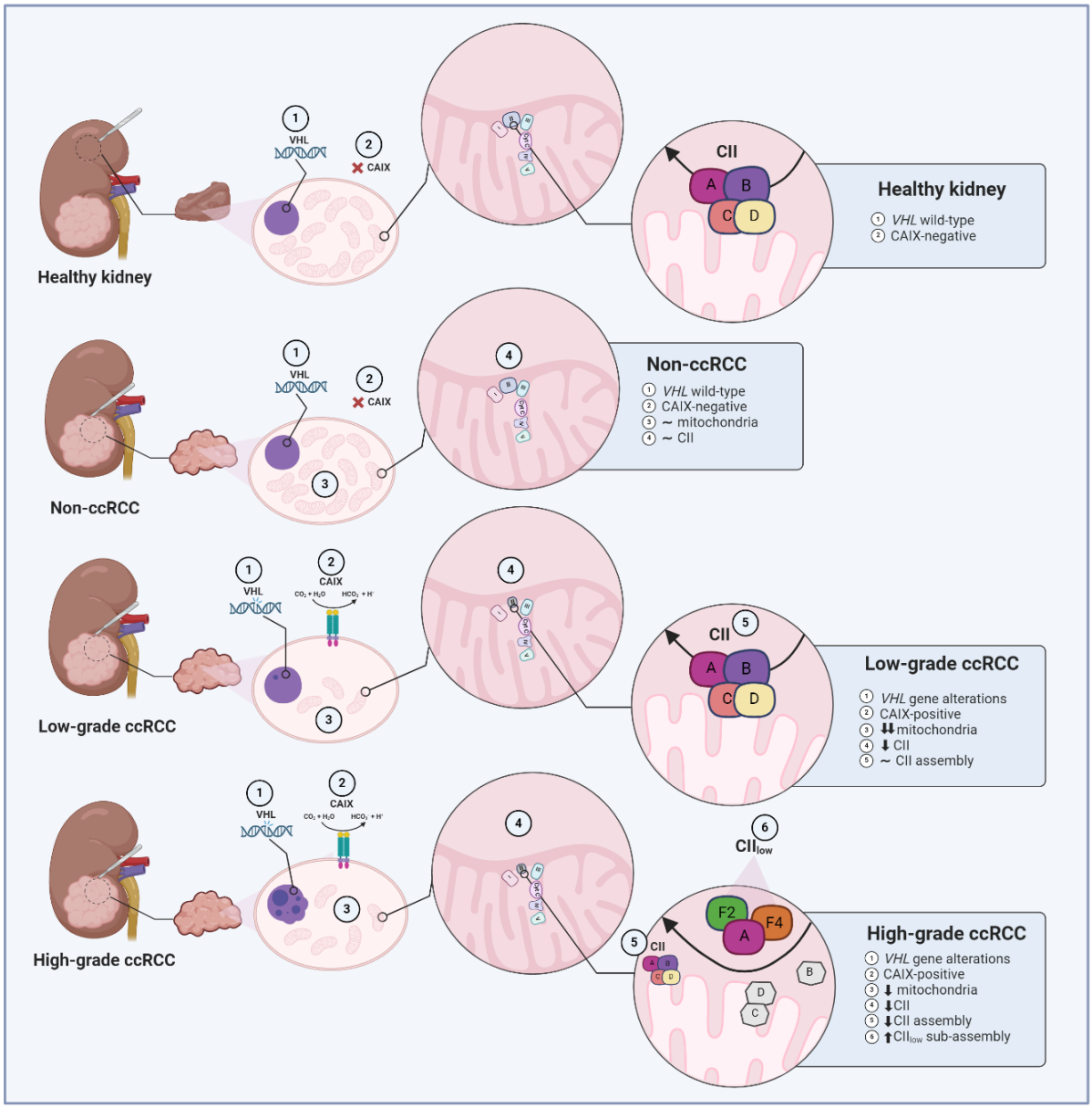

Figure 10: Mitochondrial function and CII activity across RCC subtypes and grades. This graphical abstract illustrates the differences in mitochondrial function and CII activity between normal kidney tissue and various subtypes of RCC, including pRCC, chRCC, and oncocytoma (herein as non-ccRCC) and ccRCC, as well as across tumour grades. Adapted from [203].

CII_{low} has been recognized as a distinct subassembly of CII during the maturation of SDHA. In the second part of this study, we investigated the role of two assembly factors, SDHAF2 and SDHAF4, in SDHA maturation using hPheo1 cells derived from human pheochromocytoma.

Our results show that, although multiple pathways could contribute to SDHA maturation, a preferred pathway exists in which SDHA progresses via at least three metastable intermediates: SDHA-AF2, SDHA-AF2-AF4, and SDHA-AF4. Both SDHA and SDHA-AF2 migrate as a ~100 kDa species on BN-PAGE. Previous studies have noted a specific increase in this ~100 kDa band under various physiological conditions. For instance, earlier work found that SDHA migrated as a ~100 kDa species in hypoxia or acidified environment induced by anticancer drugs. It was concluded that the species was an SDHA-SDHB heterodimer, but SDHB was not directly determined [204].

More recently, a study linked macrophage activation to CII disassembly and the appearance of an SDHA-containing species, although no more information about the presence of assembly factors or any other components was provided [205].

In subsequent research, similar SDHA-containing species were observed in cells where CII assembly was disrupted, and these species were referred to as CII_{low}. Although the identity of the species was not fully resolved at the time, one possibility was that the 100 kDa species was a complex between SDHA and assembly factor(s). There was evidence for SDHA, SDHAF2, and SDHAF4, but it was not clear whether they existed as monomers or formed homo- or heterodimers [114].

Our findings reveal that the previously proposed ~100 kDa CII_{low} species predominantly consists of SDHA and SDHAF2. Importantly, SDHA-AF2 is highly stable, the protein complex remains intact and dissociates only upon denaturation. Based on these observations, we propose that the species that most significantly accumulates as the ~100 kDa is likely the SDHA-AF2 assembly intermediate.

Additionally, we found that SDHAF4 facilitates the release of SDHAF2 from the SDHA-AF2 complex and that structural changes are key for this transition. Mechanistically, these changes appear to involve alterations in intrinsic disorder, i.e. regions lacking secondary structure, that induce allosteric shifts in SDHA. As a consequence, the binding surface of SDHA is modified to interact with SDHAF2, SDHAF2 and SDHB. In SDHAF2, the changes in intrinsic disorder

occur in regions critical for SDHA maturation. Isolated SDHAF4 is fully disordered, and binding to SDHA still preserves a large portion of the protein disordered. This intrinsic disorder is important in protein assembly, expanding known functions of intrinsic disorders. A schematic of the main findings of this study is illustrated in Figure 11.

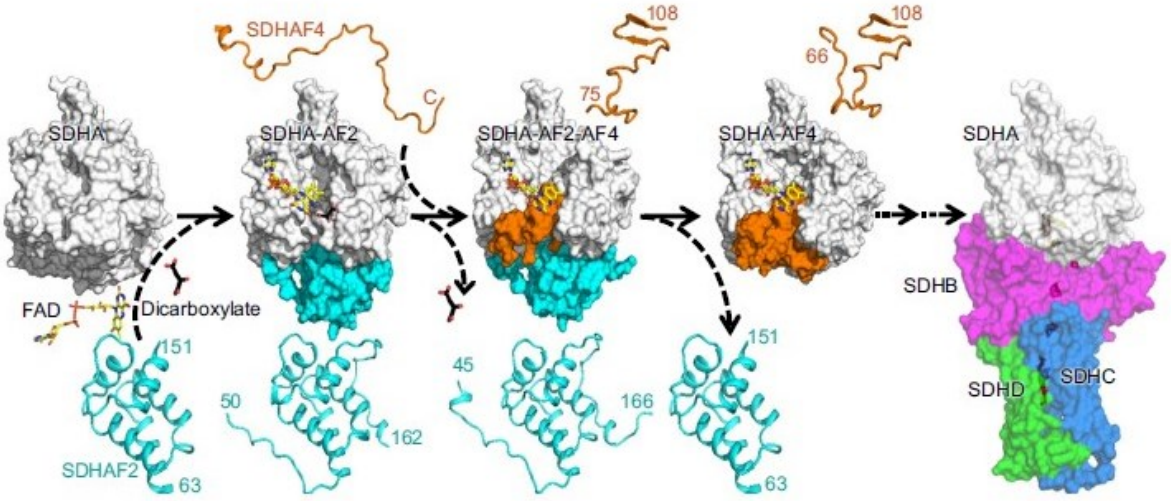

Figure 11: Model of SDHA maturation pathway. This model illustrates the preferred maturation pathway of SDHA during CII biogenesis. Assembly intermediates: SDHA-AF2, SDHA-AF2-AF4, and SDHA-AF4, as well as FAD and dicarboxylate (succinate) molecules, are shown to depict the maturation process. Adapted from [206].

Importantly, a previous study proposed the lack of enzymatic activity of the CII_{low} species [207]. This is consistent with our findings, where SDH activity was only detected in fully assembled CII with intact catalytic activity. The CII_{low} species may be therefore used to store SDHA, which could then be used for CII assembly, ensuring efficient CII assembly when needed.

In summary, our research defines the function of SDHAF2 and SDHFA4 in CII assembly. We demonstrate that SDHAF2 is essential for the flavinylation of SDHA, which is crucial for transferring electrons and reducing FAD to $FADH_2$. Additionally, we show that the role of SDHAF4 is to bind to the SDHA-SDHAF2 complex and to displace SDHAF2 to allow for further maturation of SDHA and the subsequent binding of SDHB.

In summary, we demonstrate the important role of CII in cancer, using patient samples and cell lines from two of the most common CII-associated cancers: RCC and PPGL. This work also has a translational context, particularly in light of our recent clinical trial using mitochondria-targeting anti-cancer drug MitoTam.

Although mitochondria present a promising target for therapeutic intervention, so far, only one drug targeting mitochondria, the BH3 mimetic Venetoclax, has been approved for clinical use, specifically for treating acute myeloid leukemia (AML) [208]. Our team has synthesized and evaluated various triphenylphosphonium (TPP+)-conjugated mitochondrial-targeted compounds, known as mitocans [209]. Mitotam, a modified form of tamoxifen, is conjugated with TPP+, a compound with lipophilic and positively charged properties, enabling it to easily pass through cellular membranes, including the plasma membrane and the IMM. Due to the higher membrane potential often found in cancer cells, TPP+ serves as a molecular anchor, concentrating these compounds at the IMM-matrix interface. MitoTam acts by disrupting mitochondrial CI [210].

Encouragingly, a Phase 1/1b clinical trial involving 75 patients across various cancer types (EudraCT 2017-004441-25) revealed that RCC patients experienced the greatest therapeutic benefit, with 5 out of 6 patients showing long-term stabilization or remission [211]. Notably, all five patients with benefit were diagnosed with ccRCC [212], which supports the potential relevance of MitoTam in treating ccRCC via CI destabilization. However, it also creates a concern that alterations in CII, which, as we have shown in this study, are common in ccRCC, could affect CI function. Previous studies have shown that mutations in SDH, including those in SDHA, SDHB, and SDHD, disrupt the Krebs cycle and NADH production, which are essential for CI activity [19,157,213,214]. On the other hand, CII dysfunction was found not to affect CIII or CV levels. Instead, the dual loss of SDH and CI has been identified as a key driver of the metabolic phenotype observed in SDH-mutant tumours [215]. Importantly, the loss of CI in SDH-deficient RCC has been shown to sustain the production of aspartate, critical for cell proliferation during metabolic reprogramming [213]. Understanding CII and CI dependence may therefore help guide future patient recruitment for Phase II of the clinical trial.

This research also raises important questions regarding the status of CI in different tissues affected by specific SDH mutations. For example, in Leigh syndrome and ataxia associated with SDHA mutations, CI activity remains intact [215–218]. Interestingly, studies suggest that chromaffin-derived cells, such as those in PCC/PPGL, depend on CI activity, in contrast to epithelial cell models of SDH loss, like RCC and GIST [219]. Thus, the ability to retain CI function may be a key factor in determining tissue-specific outcomes of SDH loss in cancer and neurodegenerative disorders.

Moreover, since SDHA accumulates in all CII KO states as part of the CII_{low} species, and SDHA levels remain stable under these conditions, SDHA may act as a master regulator. Our preliminary data (not shown), along with existing literature, suggests that when CII subunit genes are knocked out, except for SDHA, all other subunits are depleted, whereas SDHA itself remains highly stable. Disruption of the CII assembly machinery leads to its collapse, but SDHA is only significantly affected when its expression is aberrant. This phenomenon is particularly evident in SDH-deficient RCCs, where tumours with mutations in SDHB, SDHC, or SDHD are negative for SDHB but positive for SDHA, while SDHA-deficient RCCs are negative for both SDHA and SDHB [220]. These findings suggest that SDHA plays a pivotal role in CII assembly and stability and may serve as a critical biomarker for assessing tumourigenesis in SDH-deficient RCC.

Despite the progress made in understanding the biology of CII as discussed in this work, several unresolved questions from our study require further exploration. Key areas of study include the impact of CII changes on tumour sensitivity to MitoTam treatment, how CII, CI, and overall mitochondrial content change during metastasis and whether MitoTam treatment exerts distinct effects on primary vs. metastatic tumours. Additionally, the role of CII_{low} in circulating tumour cells and metastatic tissue requires further clarification.

6. Conclusion

CII plays a key role in mitochondrial function by connecting the TCA cycle and ETC, while also regulating metabolites involved in cellular signalling. Its important role in maintaining cellular metabolism makes it crucial for both health and disease. Disruptions to CII have been linked to the development of various cancers. This research highlights the importance of understanding the role of CII in cancer biology.

A detailed analysis of CII in RCC, particularly ccRCC, highlights the role of CII in the metabolic reprogramming seen in ccRCC. The findings reveal that ccRCC uniquely displays compromised mitochondrial content, function, and CII activity compared to healthy kidney tissue. This loss in CII and mitochondrial integrity is not as pronounced in other RCC subtypes, such as pRCC and chRCC, which retain mitochondrial characteristics more similar to normal kidney tissue. High-grade tumour cells accumulate mitochondria and show increased levels of $CIII_{low}$ subassembly. This differential mitochondrial profile, alongside specific genetic and proteomic alterations, points to a distinct pathway of metabolic adaptation in ccRCC, which supports its aggressiveness and may have implications for therapy selection.

Additionally, we show that CII assembly is crucial for the function of the protein, and SDHA maturation proceeds selectively via distinct steps of different SDHA-containing species. Through the analysis of cell lines, this research highlights the critical nature of SDHAF2 and SDHAF4 in the sequential assembly of CII.

In translational terms, the study suggests potential biomarkers, such as CII_{low}, that could serve as diagnostic indicators or therapeutic targets in RCC. Notably, the appearance of this subassembly, in high-grade ccRCC points to CII's potential role as a prognostic marker in aggressive tumour phenotypes. Additionally, the study discusses the relevance of mitochondria-targeting therapeutics, such as MitoTam, and the implications of CII deficiencies on the efficacy of these therapies in ccRCC patients, supporting the idea of a patient-specific approach based on mitochondrial profiles.

7. References

[1] Suomalainen, A., and Nunnari, J., 2024, Mitochondria at the Crossroads of Health and Disease, *Cell*, **187**(11), pp. 2601–2627.

[2] Chandel, N. S., 2014, Mitochondria as Signaling Organelles, *BMC Biol*, **12**.

[3] Chen, W., Zhao, H., and Li, Y., 2023, Mitochondrial Dynamics in Health and Disease: Mechanisms and Potential Targets, *Signal Transduct Target Ther*, **8**(1).

[4] Smith, D. R., and Keeling, P. J., 2015, Mitochondrial and Plastid Genome Architecture: Reoccurring Themes, but Significant Differences at the Extremes, *Proc Natl Acad Sci U S A*, **112**(33), pp. 10177–10184.

[5] Cogliati, S., Enriquez, J. A., and Scorrano, L., 2016, Mitochondrial Cristae: Where Beauty Meets Functionality, *Trends Biochem Sci*, **41**(3), pp. 261–273.

[6] Iovine, J. C., Claypool, S. M., and Alder, N. N., 2021, Mitochondrial Compartmentalization: Emerging Themes in Structure and Function, *Trends Biochem Sci*, **46**(11), pp. 902–917.

[7] Edwards, R., Eaglesfield, R., and Tokatlidis, K., 2021, The Mitochondrial Intermembrane Space: The Most Constricted Mitochondrial Sub-Compartment with the Largest Variety of Protein Import Pathways, *Open Biol*, **11**(3).

[8] Martínez-Reyes, I., and Chandel, N. S., 2020, Mitochondrial TCA Cycle Metabolites Control Physiology and Disease, *Nat Commun*, **11**(1).

[9] Sullivan, L. B., Gui, D. Y., Hosios, A. M., Bush, L. N., Freinkman, E., and Vander Heiden, M. G., 2015, Supporting Aspartate Biosynthesis Is an Essential Function of Respiration in Proliferating Cells, *Cell*, **162**(3), pp. 552–563.

[10] Mullen, A. R., Wheaton, W. W., Jin, E. S., Chen, P. H., Sullivan, L. B., Cheng, T., Yang, Y., Linehan, W. M., Chandel, N. S., and Deberardinis, R. J., 2012, Reductive Carboxylation Supports Growth in Tumour Cells with Defective Mitochondria, *Nature*, **481**(7381), pp. 385–388.

[11] Kang, W., Suzuki, M., Saito, T., and Miyado, K., 2021, Emerging Role of Tca Cycle-Related Enzymes in Human Diseases, *Int J Mol Sci*, **22**(23).

[12] Vercellino, I., and Sazanov, L. A., 2022, The Assembly, Regulation and Function of the Mitochondrial Respiratory Chain, *Nat Rev Mol Cell Biol*, **23**(2), pp. 141–161.

[13] Sazanov, L. A., and Hinchliffe, P., 2006, Structure of the Hydrophilic Domain of Respiratory Complex I from Thermus Thermophilus, *Science (1979)*, **311**(5766), pp. 1430–1436.

[14] Efremov, R. G., and Sazanov, L. A., 2011, Structure of the Membrane Domain of Respiratory Complex i, *Nature*, **476**(7361), pp. 414–421.

[15] Urra, F. A., Muñoz, F., Lovy, A., and Cárdenas, C., 2017, The Mitochondrial Complex(I)Ty of Cancer, *Front Oncol*, **7**(JUN).

[16] Wikström, M., and Hummer, G., 2012, Stoichiometry of Proton Translocation by Respiratory Complex I and Its Mechanistic Implications, *Proc Natl Acad Sci U S A*, **109**(12), pp. 4431–4436.

[17] Bezawork-Geleta, A., Rohlena, J., Dong, L., Pacak, K., and Neuzil, J., 2017, Mitochondrial Complex II: At the Crossroads, *Trends Biochem Sci*, **42**(4), pp. 312–325.

[18] Alcázar-Fabra, M., Rodríguez-Sánchez, F., Trevisson, E., and Brea-Calvo, G., 2021, Primary Coenzyme Q Deficiencies: A Literature Review and Online Platform of Clinical Features to Uncover Genotype-Phenotype Correlations, *Free Radic Biol Med*, **167**, pp. 141–180.

[19] Čunátová, K., and Fernández-Vizarra, E., 2024, Pathological Variants in Nuclear Genes Causing Mitochondrial Complex III Deficiency: An Update, *J Inherit Metab Dis*.

[20] Kadenbach, B., and Hüttemann, M., 2015, The Subunit Composition and Function of Mammalian Cytochrome c Oxidase, *Mitochondrion*, **24**, pp. 64–76.

[21] Jonckheere, A. I., Smeitink, J. A. M., and Rodenburg, R. J. T., 2012, Mitochondrial ATP Synthase: Architecture, Function and Pathology, *J Inherit Metab Dis*, **35**(2), pp. 211–225.

[22] Arnold, P. K., and Finley, L. W. S., 2023, Regulation and Function of the Mammalian Tricarboxylic Acid Cycle, *Journal of Biological Chemistry*, **299**(2).

[23] Chance, B., Williams, G. R., and Hollunger, R., 1963, *Nature of Intramolecularly Assisted Ionization: Methyl Substitution on a 5,6-Double Bond*.
[24] Hackenbrock, C. R., Chazotte, B., and Shaila Gupte, S., 1986, *The Random Collision Model and a Critical Assessment of Diffusion and Collision in Mitochondrial Electron Transport*.
[25] Milenkovic, D., Blaza, J. N., Larsson, N. G., and Hirst, J., 2017, The Enigma of the Respiratory Chain Supercomplex, *Cell Metab*, **25**(4), pp. 765–776.
[26] Gu, J., Wu, M., Guo, R., Yan, K., Lei, J., Gao, N., and Yang, M., 2016, The Architecture of the Mammalian Respirasome, *Nature*, **537**(7622), pp. 639–643.
[27] Letts, J. A., Fiedorczuk, K., and Sazanov, L. A., 2016, The Architecture of Respiratory Supercomplexes, *Nature*, **537**(7622), pp. 644–648.
[28] Wu, M., Gu, J., Guo, R., Huang, Y., and Yang, M., 2016, Structure of Mammalian Respiratory Supercomplex I1III2IV1, *Cell*, **167**(6), pp. 1598-1609.e10.
[29] Guo, R., Zong, S., Wu, M., Gu, J., and Yang, M., 2017, Architecture of Human Mitochondrial Respiratory Megacomplex I2III2IV2, *Cell*, **170**(6), pp. 1247-1257.e12.
[30] Caruana, N. J., and Stroud, D. A., 2020, The Road to the Structure of the Mitochondrial Respiratory Chain Supercomplex, *Biochem Soc Trans*, **48**(2), pp. 621–629.
[31] Mühleip, A., Flygaard, R. K., Baradaran, R., Haapanen, O., Gruhl, T., Tobiasson, V., Maréchal, A., Sharma, V., and Amunts, A., 2023, Structural Basis of Mitochondrial Membrane Bending by the I–II–III2–IV2 Supercomplex, *Nature*, **615**(7954), pp. 934–938.
[32] Burger, G., Lang, B. F., Reitht, M., and Gray, M. W., 1996, *Genes Encoding the Same Three Subunits of Respiratory Complex II Are Present in the Mitochondrial DNA of Two Phylogenetically Distant Eukaryotes (Mitochondrion/Succinate:Ubiquinone Oxidoreductase/Succinate Dehydrogenase/Porphyra Purpurea/Reclinomonas Americana)*. [Online]. Available: http://megasun.bch.umontreal.ca/.
[33] Wiedemann, N., and Pfanner, N., 2024, Mitochondrial Machineries for Protein Import and Assembly, **6**, p. 30.
[34] Esteban-Amo, M. J., Jiménez-Cuadrado, P., Serrano-Lorenzo, P., de la Fuente, M. Á., and Simarro, M., 2024, Succinate Dehydrogenase and Human Disease: Novel Insights into a Well-Known Enzyme, *Biomedicines*, **12**(9), p. 2050.
[35] Du, Z., Zhou, X., Lai, Y., Xu, J., Zhang, Y., Zhou, S., Feng, Z., Yu, L., Tang, Y., Wang, W., Yu, L., Tian, C., Ran, T., Chen, H., Guddat, L. W., Liu, F., Gao, Y., Rao, Z., and Gong, H., 2023, Structure of the Human Respiratory Complex II, *Proc Natl Acad Sci U S A*, **120**(118).
[36] Moosavi, B., Berry, E. A., Zhu, X. L., Yang, W. C., and Yang, G. F., 2019, The Assembly of Succinate Dehydrogenase: A Key Enzyme in Bioenergetics, *Cellular and Molecular Life Sciences*, **76**(20), pp. 4023–4042.
[37] McNeil, M. B., Hampton, H. G., Hards, K. J., Watson, B. N. J., Cook, G. M., and Fineran, P. C., 2014, The Succinate Dehydrogenase Assembly Factor, SdhE, Is Required for the Flavinylation and Activation of Fumarate Reductase in Bacteria, *FEBS Lett*, **588**(3), pp. 414–421.
[38] Zafreen, L., Walker-Kopp, N., Huang, L. S., and Berry, E., 2016, In-Vitro, SDH5-Dependent Flavinylation of Immobilized Human Respiratory Complex II Flavoprotein, *Arch Biochem Biophys*, **604**, pp. 47–56.
[39] Maklashina, E., Rajagukguk, S., Starbird, C. A., McDonald, W. H., Koganitsky, A., Eisenbach, M., Iverson, T. M., and Cecchini, G., 2016, Binding of the Covalent Flavin Assembly Factor to the Flavoprotein Subunit of Complex II, *Journal of Biological Chemistry*, **291**(6), pp. 2904–2916.
[40] Sharma, P., Maklashina, E., Cecchini, G., and Iverson, T. M., 2020, The Roles of SDHAF2 and Dicarboxylate in Covalent Flavinylation of SDHA, the Human Complex II Flavoprotein, **117**, pp. 23548–23556.
[41] Kounosu, A., 2014, Analysis of Covalent Flavinylation Using Thermostable Succinate Dehydrogenase from Thermus Thermophilus and Sulfolobus Tokodaii Lacking SdhE Homologs, *FEBS Lett*, **588**(6), pp. 1058–1063.

[42] Bezawork-Geleta, A., Dong, L., Rohlena, J., and Neuzil, J., 2016, The Assembly Factor SDHAF2 Is Dispensable for Flavination of the Catalytic Subunit of Mitochondrial Complex II in Breast Cancer Cells, *Journal of Biological Chemistry*, **291**(41), pp. 21414–21420.
[43] Maklashina, E., Iverson, T. M., and Cecchini, G., 2022, How an Assembly Factor Enhances Covalent FAD Attachment to the Flavoprotein Subunit of Complex II, *Journal of Biological Chemistry*, **298**(10).
[44] Chen, C. L., Ishihara, T., Pal, S., Huang, W. L., Ogasawara, E., Chang, C. R., and Ishihara, N., 2024, SDHAF2 Facilitates Mitochondrial Respiration through Stabilizing Succinate Dehydrogenase and Cytochrome c Oxidase Assemblies, *Mitochondrion*, **79**.
[45] Zhou, Z., Ma, A., Moore, T. M., Wolf, D. M., Yang, N., Tran, P., Segawa, M., Strumwasser, A. R., Ren, W., Fu, K., Wanagat, J., van der Bliek, A. M., Crosbie-Watson, R., Liesa, M., Stiles, L., Acin-Perez, R., Mahata, S., Shirihai, O., Goodarzi, M. O., Handzlik, M., Metallo, C. M., Walker, D. W., and Hevener, A. L., 2024, Drp1 Controls Complex II Assembly and Skeletal Muscle Metabolism by Sdhaf2 Action on Mitochondria, *Sci Adv*, **10**(14).
[46] Wang, X., Zhang, X., Cao, K., Zeng, M., Fu, X., Zheng, A., Zhang, F., Gao, F., Zou, X., Li, H., Li, M., Lv, W., Xu, J., Long, J., Zang, W., Chen, J., Ding, J., Liu, J., and Feng, Z., 2022, Cardiac Disruption of SDHAF4-Mediated Mitochondrial Complex II Assembly Promotes Dilated Cardiomyopathy, *Nat Commun*, **13**(1).
[47] Van Vranken, J. G., Bricker, D. K., Dephoure, N., Gygi, S. P., Cox, J. E., Thummel, C. S., and Rutter, J., 2014, SDHAF4 Promotes Mitochondrial Succinate Dehydrogenase Activity and Prevents Neurodegeneration, *Cell Metab*, **20**(2), pp. 241–252.
[48] Rouault, T. A., 2012, Biogenesis of Iron-Sulfur Clusters in Mammalian Cells: New Insights and Relevance to Human Disease, *DMM Disease Models and Mechanisms*, **5**(2), pp. 155–164.
[49] Maio, N., Singh, A., Uhrigshardt, H., Saxena, N., Tong, W. H., and Rouault, T. A., 2014, Cochaperone Binding to LYR Motifs Confers Specificity of Iron Sulfur Cluster Delivery, *Cell Metab*, **19**(3), pp. 445–457.
[50] Maio, N., and Rouault, T. A., 2016, Mammalian Fe-S Proteins: Definition of a Consensus Motif Recognized by the Co-Chaperone HSC20, *Metallomics*, **8**(10), pp. 1032–1046.
[51] Meyer, E. H., Welchen, E., and Carrie, C., 2024, Assembly of the Complexes of the Oxidative Phosphorylation System in Land Plant Mitochondria, *Annu. Rev. Plant Biol*, **70**, p. 29.
[52] Przybyla-Toscano, J., Christ, L., Keech, O., and Rouhier, N., 2021, Iron-Sulfur Proteins in Plant Mitochondria: Roles and Maturation, *J Exp Bot*, **72**(6), pp. 2014–2044.
[53] Na, U., Yu, W., Cox, J., Bricker, D. K., Brockmann, K., Rutter, J., Thummel, C. S., and Winge, D. R., 2014, The LYR Factors Sdhaf1 and SDHAF3 Mediate Maturation of the Iron-Sulfur Subunit of Succinate Dehydrogenase, *Cell Metab*, **20**(2), pp. 253–266.
[54] Ghezzi, D., Goffrini, P., Uziel, G., Horvath, R., Klopstock, T., Lochmüller, H., D'Adamo, P., Gasparini, P., Strom, T. M., Prokisch, H., Invernizzi, F., Ferrero, I., and Zeviani, M., 2009, SDHAF1, Encoding a LYR Complex-II Specific Assembly Factor, Is Mutated in SDH-Defective Infantile Leukoencephalopathy, *Nat Genet*, **41**(6), pp. 654–656.
[55] Sun, F., Huo, X., Zhai, Y., Wang, A., Xu, J., Su, D., Bartlam, M., and Rao, Z., 2005, Crystal Structure of Mitochondrial Respiratory Membrane Protein Complex II, *Cell*, **121**(7), pp. 1043–1057.
[56] Horsefield, R., Yankovskaya, V., Sexton, G., Whittingham, W., Shiomi, K., Omura, S., Byrne, B., Cecchini, G., and Iwata, S., 2006, Structural and Computational Analysis of the Quinone-Binding Site of Complex II (Succinate-Ubiquinone Oxidoreductase): A Mechanism of Electron Transfer and Proton Conduction during Ubiquinone Reduction, *Journal of Biological Chemistry*, **281**(11), pp. 7309–7316.
[57] Cao, W., Wang, X., Lv, W., Zou, X., Xu, J., Cao, K., Liu, J., and Feng, Z., *Assembly of Mitochondrial Succinate Dehydrogenase in Human Health and Disease*.
[58] Robinson, K. M., and Lemire, B. D., 1996, *Covalent Attachment of FAD to the Yeast Succinate Dehydrogenase Flavoprotein Requires Import into Mitochondria, Presequence Removal, and Folding**.
[59] Yoshida, S., Tsutsumi, S., Muhlebach, G., Sourbier, C., Lee, M. J., Lee, S., Vartholomaiou, E., Tatokoro, M., Beebe, K., Miyajima, N., Mohney, R. P., Chen, Y., Hasumi, H., Xu, W., Fukushima, H.,

Nakamura, K., Koga, F., Kihara, K., Trepel, J., Picard, D., and Neckers, L., 2013, Molecular Chaperone TRAP1 Regulates a Metabolic Switch between Mitochondrial Respiration and Aerobic Glycolysis, *Proc Natl Acad Sci U S A*, **110**(17).

[60] Sciacovelli, M., Guzzo, G., Morello, V., Frezza, C., Zheng, L., Nannini, N., Calabrese, F., Laudiero, G., Esposito, F., Landriscina, M., Defilippi, P., Bernardi, P., and Rasola, A., 2013, The Mitochondrial Chaperone TRAP1 Promotes Neoplastic Growth by Inhibiting Succinate Dehydrogenase, *Cell Metab*, **17**(6), pp. 988–999.

[61] Guzzo, G., Sciacovelli, M., Bernardi, P., and Rasola, A., *Inhibition of Succinate Dehydrogenase by the Mitochondrial Chaperone TRAP1 Has Anti-Oxidant and Anti-Apoptotic Effects on Tumor Cells*. [Online]. Available: www.impactjournals.com/oncotarget/.

[62] Zhao, X., Sun, K., Lan, Z., Song, W., Cheng, L., Chi, W., Chen, J., Huo, Y., Xu, L., Liu, X., Deng, H., Siegenthaler, J. A., and Chen, L., 2017, Tenofovir and Adefovir Down-Regulate Mitochondrial Chaperone TRAP1 and Succinate Dehydrogenase Subunit B to Metabolically Reprogram Glucose Metabolism and Induce Nephrotoxicity, *Sci Rep*, **7**.

[63] Joshi, A., Dai, L., Liu, Y., Lee, J., Ghahhari, N. M., Segala, G., Beebe, K., Jenkins, L. M., Lyons, G. C., Bernasconi, L., Tsai, F. T. F., Agard, D. A., Neckers, L., and Picard, D., 2020, The Mitochondrial HSP90 Paralog TRAP1 Forms an OXPHOS-Regulated Tetramer and Is Involved in Mitochondrial Metabolic Homeostasis, *BMC Biol*, **18**(1).

[64] Cimen, H., Han, M. J., Yang, Y., Tong, Q., Koc, H., and Koc, E. C., 2010, Regulation of Succinate Dehydrogenase Activity by SIRT3 in Mammalian Mitochondria, *Biochemistry*, **49**(2), pp. 304–311.

[65] Park, J., Chen, Y., Tishkoff, D. X., Peng, C., Tan, M., Dai, L., Xie, Z., Zhang, Y., Zwaans, B. M. M., Skinner, M. E., Lombard, D. B., and Zhao, Y., 2013, SIRT5-Mediated Lysine Desuccinylation Impacts Diverse Metabolic Pathways, *Mol Cell*, **50**(6), pp. 919–930.

[66] Kumar, R., Landry, A. P., Guha, A., Vitvitsky, V., Lee, H. J., Seike, K., Reddy, P., Lyssiotis, C. A., and Banerjee, R., 2022, A Redox Cycle with Complex II Prioritizes Sulfide Quinone Oxidoreductase Dependent H2S Oxidation, *Journal of Biological Chemistry*, **298**(1).

[67] Spinelli, J. B., Rosen, P. C., Sprenger, H.-G., Puszynska, A. M., Mann, J. L., Roessler, J. M., Cangelosi, A. L., Henne, A., Condon, K. J., Zhang, T., Kunchok, T., Lewis, C. A., Chandel, N. S., and Sabatini, D. M., *Fumarate Is a Terminal Electron Acceptor in the Mammalian Electron Transport Chain*. [Online]. Available: https://www.science.org.

[68] Boxma, B., deGraaf, R. M., and van der Staay, G. W. M., 2005, An Anaerobic Mitochondrion Produces Hydrogen, *Nature*, **434**(7029), pp. 72–74.

[69] Robb, E. L., Hall, A. R., Prime, T. A., Eaton, S., Szibor, M., Viscomi, C., James, A. M., and Murphy, M. P., 2018, Control of Mitochondrial Superoxide Production by Reverse Electron Transport at Complex I, *Journal of Biological Chemistry*, **293**(25), pp. 9869–9879.

[70] Bonke, E., Zwicker, K., and Dröse, S., 2015, Manganese Ions Induce H2O2 Generation at the Ubiquinone Binding Site of Mitochondrial Complex II, *Arch Biochem Biophys*, **580**, pp. 75–83.

[71] Sies, H., 2017, Hydrogen Peroxide as a Central Redox Signaling Molecule in Physiological Oxidative Stress: Oxidative Eustress, *Redox Biol*, **11**, pp. 613–619.

[72] Cadenas, S., 2018, Mitochondrial Uncoupling, ROS Generation and Cardioprotection, *Biochim Biophys Acta Bioenerg*, **1859**(9), pp. 940–950.

[73] Dong, L. F., Low, P., Dyason, J. C., Wang, X. F., Prochazka, L., Witting, P. K., Freeman, R., Swettenham, E., Valis, K., Liu, J., Zobalova, R., Turanek, J., Spitz, D. R., Domann, F. E., Scheffler, I. E., Ralph, S. J., and Neuzil, J., 2008, α-Tocopheryl Succinate Induces Apoptosis by Targeting Ubiquinone-Binding Sites in Mitochondrial Respiratory Complex II, *Oncogene*, **27**(31), pp. 4324–4335.

[74] De Castro Fonseca, M., Aguiar, C. J., Da Rocha Franco, J. A., Gingold, R. N., and Leite, M. F., 2016, GPR91: Expanding the Frontiers of Krebs Cycle Intermediates, *Cell Communication and Signaling*, **14**(1).

[75] Mu, X., Zhao, T., Xu, C., Shi, W., Geng, B., Shen, J., Zhang, C., Pan, J., Yang, J., Hu, S., Lv,

Y., Wen, H., and You, Q., *Oncometabolite Succinate Promotes Angiogenesis by Upregulating VEGF Expression through GPR91-Mediated STAT3 and ERK Activation*. [Online]. Available: www.impactjournals.com/oncotarget.

[76] Wu, J. Y., Huang, T. W., Hsieh, Y. T., Wang, Y. F., Yen, C. C., Lee, G. L., Yeh, C. C., Peng, Y. J., Kuo, Y. Y., Wen, H. T., Lin, H. C., Hsiao, C. W., Wu, K. K., Kung, H. J., Hsu, Y. J., and Kuo, C. C., 2020, Cancer-Derived Succinate Promotes Macrophage Polarization and Cancer Metastasis via Succinate Receptor, *Mol Cell*, **77**(2), pp. 213-227.e5.

[77] Tannahill, G. M., Curtis, A. M., Adamik, J., Palsson-Mcdermott, E. M., McGettrick, A. F., Goel, G., Frezza, C., Bernard, N. J., Kelly, B., Foley, N. H., Zheng, L., Gardet, A., Tong, Z., Jany, S. S., Corr, S. C., Haneklaus, M., Caffrey, B. E., Pierce, K., Walmsley, S., Beasley, F. C., Cummins, E., Nizet, V., Whyte, M., Taylor, C. T., Lin, H., Masters, S. L., Gottlieb, E., Kelly, V. P., Clish, C., Auron, P. E., Xavier, R. J., and O'Neill, L. A. J., 2013, Succinate Is an Inflammatory Signal That Induces IL-1β through HIF-1α, *Nature*, **496**(7444), pp. 238–242.

[78] Gudgeon, N., Munford, H., Bishop, E. L., Hill, J., Fulton-Ward, T., Bending, D., Roberts, J., Tennant, D. A., and Dimeloe, S., 2022, Succinate Uptake by T Cells Suppresses Their Effector Function via Inhibition of Mitochondrial Glucose Oxidation, *Cell Rep*, **40**(7).

[79] Losman, J. A., Koivunen, P., and Kaelin, W. G., 2020, 2-Oxoglutarate-Dependent Dioxygenases in Cancer, *Nat Rev Cancer*, **20**(12), pp. 710–726.

[80] Selak, M. A., Armour, S. M., MacKenzie, E. D., Boulahbel, H., Watson, D. G., Mansfield, K. D., Pan, Y., Simon, M. C., Thompson, C. B., and Gottlieb, E., 2005, Succinate Links TCA Cycle Dysfunction to Oncogenesis by Inhibiting HIF-α Prolyl Hydroxylase, *Cancer Cell*, **7**(1), pp. 77–85.

[81] Schito, L., and Semenza, G. L., 2016, Hypoxia-Inducible Factors: Master Regulators of Cancer Progression, *Trends Cancer*, **2**(12), pp. 758–770.

[82] Hoekstra, A. S., De Graaff, M. A., Briaire-De Bruijn, I. H., Seifar, R. M., Van Minderhout, I., Cornelisse, C. J., Hogendoorn, P. C. W., Breuning, M. H., Suijker, J., Korpershoek, E., Kunst, H. P. M., Frizzell, N., Devilee, P., Bayley, J.-P., and Bovée, J. V. M. G., 2015, *Inactivation of SDH and FH Cause Loss of 5hmC and Increased H3K9me3 in Paraganglioma/Pheochromocytoma and Smooth Muscle Tumors*. [Online]. Available: www.impactjournals.com/oncotarget.

[83] Sulkowski, P. L., Sundaram, R. K., Oeck, S., Corso, C. D., Liu, Y., Noorbakhsh, S., Niger, M., Boeke, M., Ueno, D., Kalathil, A. N., Bao, X., Li, J., Shuch, B., Bindra, R. S., and Glazer, P. M., 2018, Krebs-Cycle-Deficient Hereditary Cancer Syndromes Are Defined by Defects in Homologous-Recombination DNA Repair, *Nat Genet*, **50**(8), pp. 1086–1092.

[84] Pang, Y., Lu, Y., Caisova, V., Liu, Y., Bullova, P., Huynh, T. T., Zhou, Y., Yu, D., Frysak, Z., Hartmann, I., Taieb, D., Pacak, K., and Yang, C., 2018, Targeting NADþ/PARP DNA Repair Pathway as a Novel Therapeutic Approach to SDHB-Mutated Cluster I Pheochromocytoma and Paraganglioma, *Clinical Cancer Research*, **24**(14), pp. 3423–3432.

[85] Parfait, B., Chretien, D., Rötig, A., Marsac, C., Munnich, A., and Rustin, P., 2000, Compound Heterozygous Mutations in the Flavoprotein Gene of the Respiratory Chain Complex II in a Patient with Leigh Syndrome, *Hum Genet*, **106**(2), pp. 236–243.

[86] Kent, J. D., Klug, L. R., and Heinrich, M. C., 2024, A Novel Human *SDHA* -Knockout Cell Line Model for the Functional Analysis of Clinically Relevant SDHA Variants, *Clinical Cancer Research*, pp. OF1–OF14.

[87] Tung, M. L., Chandra, B., Dillahunt, K., Gosse, M. D., Sato, T. S., and Sidhu, A., 2022, Co-Occurrence of VHL and SDHA Pathogenic Variants: A Case Report, *Front Oncol*, **12**.

[88] Ruhoy, I. S., and Saneto, R. P., 2014, The Genetics of Leigh Syndrome and Its Implications for Clinical Practice and Risk Management, *Application of Clinical Genetics*, **7**, pp. 221–234.

[89] Jain-Ghai, S., Cameron, J. M., Al Maawali, A., Blaser, S., Mackay, N., Robinson, B., and Raiman, J., 2013, Complex II Deficiency-A Case Report and Review of the Literature, *Am J Med Genet A*, **161**(2), pp. 285–294.

[90] Goetzman, E., Gong, Z., Zhang, B., and Muzumdar, R., 2023, Complex II Biology in Aging, Health, and Disease, *Antioxidants*, **12**(7).

[91] Wojtovich, A. P., Smith, C. O., Haynes, C. M., Nehrke, K. W., and Brookes, P. S., 2013, Physiological Consequences of Complex II Inhibition for Aging, Disease, and the MKATP Channel, *Biochim Biophys Acta Bioenerg*, **1827**(5), pp. 598–611.
[92] Long, J., He, P., Shen, Y., and Li, R., 2012, New Evidence of Mitochondria Dysfunction in the Female Alzheimer's Disease Brain: Deficiency of Estrogen Receptor-β, *Journal of Alzheimer's Disease*, **30**(3), pp. 545–558.
[93] Chi, J., Xie, Q., Jia, J., Liu, X., Sun, J., Deng, Y., and Yi, L., 2018, Integrated Analysis and Identification of Novel Biomarkers in Parkinson's Disease, *Front Aging Neurosci*, **10**(JUN).
[94] Al Khazal, F., Kang, S., Nelson Holte, M., Choi, D. S., Singh, R., Ortega-Sáenz, P., López-Barneo, J., and Maher, L. J., 2021, Unexpected Obesity, Rather than Tumorigenesis, in a Conditional Mouse Model of Mitochondrial Complex II Deficiency, *FASEB Journal*, **35**(2).
[95] Harada, K., Yahata, T., Onizuka, M., Ishii, T., Aziz Ibrahim, A., Kikkawa, E., Gondo, Y., and Ando, K., 2023, Mitochondrial Electron Transport Chain Complex II Dysfunction Causes Premature Aging of Hematopoietic Stem Cells, *Stem Cells*, **41**(1), pp. 39–49.
[96] Pitsava, G., Settas, N., Faucz, F. R., and Stratakis, C. A., 2021, Carney Triad, Carney-Stratakis Syndrome, 3PAS and Other Tumors Due to SDH Deficiency, *Front Endocrinol (Lausanne)*, **12**.
[97] Cao, Z., Song, J. H., Kang, Y. W., Yoon, J. H., Nam, S. W., Lee, J. Y., and Park, W. S., 2010, Analysis of Succinate Dehydrogenase Subunit B Gene Alterations in Gastric Cancers, *Pathol Int*, **60**(8), pp. 559–565.
[98] Baysal, B. E., 2007, A Recurrent Stop-Codon Mutation in Succinate Dehydrogenase Subunit B Gene in Normal Peripheral Blood and Childhood T-Cell Acute Leukemia, *PLoS One*, **2**(5).
[99] Grau, E., Oltra, S., Orellana, C., Hernández-Martí, M., Castel, V., and Martínez, F., There Is No Evidence That the SDHB Gene Is Involved in Neuroblastoma Development, *Oncol Res*, **15**, pp. 393–398. [Online]. Available: www.cognizantcommunication.com.
[100] Janeway, K. A., and Pappo, A., 2012, *Treatment Guidelines for Gastrointestinal Stromal Tumors in Children and Young Adults*. [Online]. Available: www.jpho-online.com.
[101] Corless, C. L., 2014, Gastrointestinal Stromal Tumors: What Do We Know Now?, *Modern Pathology*, **27**(SUPPL. 1).
[102] Miettinen, M., and Lasota, J., 2014, Succinate Dehydrogenase Deficient Gastrointestinal Stromal Tumors (GISTs) - A Review, *International Journal of Biochemistry and Cell Biology*, **53**, pp. 514–519.
[103] Killian, J. K., Kim, S. Y., Miettinen, M., Smith, C., Merino, M., Tsokos, M., Quezado, M., Smith, W. I., Jahromi, M. S., Xekouki, P., Szarek, E., Walker, R. L., Lasota, J., Raffeld, M., Klotzle, B., Wang, Z., Jones, L., Zhu, Y., Wang, Y., Waterfall, J. J., O'Sullivan, M. J., Bibikova, M., Pacak, K., Stratakis, C., Janeway, K. A., Schiffman, J. D., Fan, J. B., Helman, L., and Meltzer, P. S., 2013, Succinate Dehydrogenase Mutation Underlies Global Epigenomic Divergence in Gastrointestinal Stromal Tumor, *Cancer Discov*, **3**(6), pp. 648–657.
[104] Mete, O., Asa, S. L., Gill, A. J., Kimura, N., de Krijger, R. R., and Tischler, A., 2022, Overview of the 2022 WHO Classification of Paragangliomas and Pheochromocytomas, *Endocr Pathol*, **33**(1), pp. 90–114.
[105] Lenders, J. W. M., Kerstens, M. N., Amar, L., Prejbisz, A., Robledo, M., Taieb, D., Pacak, K., Crona, J., Zelinka, T., Mannelli, M., Deutschbein, T., Timmers, H. J. L. M., Castinetti, F., Dralle, H., Widimský, J., Gimenez-Roqueplo, A. P., and Eisenhofer, G., 2020, Genetics, Diagnosis, Management and Future Directions of Research of Phaeochromocytoma and Paraganglioma: A Position Statement and Consensus of the Working Group on Endocrine Hypertension of the European Society of Hypertension, *J Hypertens*, **38**(8), pp. 1443–1456.
[106] Nölting, S., Bechmann, N., Taieb, D., Beuschlein, F., Fassnacht, M., Kroiss, M., Eisenhofer, G., Grossman, A., and Pacak, K., 2022, Personalized Management of Pheochromocytoma and Paraganglioma, *Endocr Rev*, **43**(2), pp. 199–239.
[107] Fishbein, L., Leshchiner, I., Walter, V., Danilova, L., Zmuda, E., et al., 2017, Comprehensive Molecular Characterization of Pheochromocytoma and Paraganglioma, *Cancer Cell*, **31**(2), pp.

181–193.
[108] Wachtel, H., and Nathanson, K. L., 2024, Molecular Genetics of Pheochromocytoma/Paraganglioma, *Curr Opin Endocr Metab Res*, **36**.
[109] Jeeyavudeen, M. S., Mathiyalagan, N., James, C. F., and Pappachan, J. M., 2024, Tumor Metabolism in Pheochromocytomas: Clinical and Therapeutic Implications, *Explor Target Antitumor Ther*, **5**(2), pp. 349–373.
[110] Jawed, I., Velarde, M., Därr, R., Wolf, K. I., Adams, K., Venkatesan, A. M., Balasubramaniam, S., Poruchynsky, M. S., Reynolds, J. C., Pacak, K., and Fojo, T., 2018, Continued Tumor Reduction of Metastatic Pheochromocytoma/Paraganglioma Harboring Succinate Dehydrogenase Subunit B Mutations with Cyclical Chemotherapy, *Cell Mol Neurobiol*, **38**(5), pp. 1099–1106.
[111] Nölting, S., Grossman, A., and Pacak, K., 2019, Metastatic Phaeochromocytoma: Spinning Towards More Promising Treatment Options, *Experimental and Clinical Endocrinology and Diabetes*, **127**(2–3), pp. 117–128.
[112] O'Kane, G. M., Ezzat, S., Joshua, A. M., Bourdeau, I., Leibowitz-Amit, R., Olney, H. J., Krzyzanowska, M., Reuther, D., Chin, S., Wang, L., Brooks, K., Hansen, A. R., Asa, S. L., and Knox, J. J., 2019, A Phase 2 Trial of Sunitinib in Patients with Progressive Paraganglioma or Pheochromocytoma: The SNIPP Trial, *Br J Cancer*, **120**(12), pp. 1113–1119.
[113] Gruber, L. M., Hart, S. N., and Maher III, L. J., 2023, Succinate Dehydrogenase Variants in Paraganglioma: Why Are B Subunit Variants "Bad"?, *Endocrine Oncology*, **3**(1).
[114] Bezawork-Geleta, A., Wen, H., Dong, L., Yan, B., Vider, J., Boukalova, S., Krobova, L., Vanova, K., Zobalova, R., Sobol, M., Hozak, P., Novais, S. M., Caisova, V., Abaffy, P., Naraine, R., Pang, Y., Zaw, T., Zhang, P., Sindelka, R., Kubista, M., Zuryn, S., Molloy, M. P., Berridge, M. V., Pacak, K., Rohlena, J., Park, S., and Neuzil, J., 2018, Alternative Assembly of Respiratory Complex II Connects Energy Stress to Metabolic Checkpoints, *Nat Commun*, **9**(1).
[115] Smith, E. H., Janknecht, R., and Maher, J. L., 2007, Succinate Inhibition of α-Ketoglutarate-Dependent Enzymes in a Yeast Model of Paraganglioma, *Hum Mol Genet*, **16**(24), pp. 3136–3148.
[116] Taïeb, D., Wanna, G. B., Ahmad, M., Lussey-Lepoutre, C., Perrier, N. D., Nölting, S., Amar, L., Timmers, H. J. L. M., Schwam, Z. G., Estrera, A. L., Lim, M., Pollom, E. L., Vitzthum, L., Bourdeau, I., Casey, R. T., Castinetti, F., Clifton-Bligh, R., Corssmit, E. P. M., de Krijger, R. R., Del Rivero, J., Eisenhofer, G., Ghayee, H. K., Gimenez-Roqueplo, A. P., Grossman, A., Imperiale, A., Jansen, J. C., Jha, A., Kerstens, M. N., Kunst, H. P. M., Liu, J. K., Maher, E. R., Marchioni, D., Mercado-Asis, L. B., Mete, O., Naruse, M., Nilubol, N., Pandit-Taskar, N., Sebag, F., Tanabe, A., Widimsky, J., Meuter, L., Lenders, J. W. M., and Pacak, K., 2023, Clinical Consensus Guideline on the Management of Phaeochromocytoma and Paraganglioma in Patients Harbouring Germline SDHD Pathogenic Variants, *Lancet Diabetes Endocrinol*, **11**(5), pp. 345–361.
[117] Rijken, J. A., Van Hulsteijn, L. T., Dekkers, O. M., Niemeijer, N. D., Leemans, C. R., Eijkelenkamp, K., Van Der Horst-Schrivers, A. N. A., Kerstens, M. N., Van Berkel, A., Timmers, H. J. L. M., Kunst, H. P. M., Bisschop, P. H. L. T., Dreijerink, K. M. A., Van Dooren, M. F., Hes, F. J., Jansen, J. C., Corssmit, E. P. M., and Hensen, E. F., 2019, Increased Mortality in SDHB but Not in SDHD Pathogenic Variant Carriers, *Cancers (Basel)*, **11**(1).
[118] Armstrong, N., Storey, C. M., Noll, S. E., Margulis, K., Soe, M. H., Xu, H., Yeh, B., Fishbein, L., Kebebew, E., Howitt, B. E., Zare, R. N., Sage, J., and Annes, J. P., 2022, SDHB Knockout and Succinate Accumulation Are Insufficient for Tumorigenesis but Dual SDHB/NF1 Loss Yields SDHx-like Pheochromocytomas, *Cell Rep*, **38**(9).
[119] Kiuru, M., and Busam, K. J., 2017, The NF1 Gene in Tumor Syndromes and Melanoma, *Laboratory Investigation*, **97**(2), pp. 146–157.
[120] Lewis, E. P., Al Khazal, F., Wilbanks, B., Gades, N. M., Ortega-Sáenz, P., López-Barneo, J., Adameyko, I., and Maher, L. J., 2024, Mouse Developmental Defects, but Not Paraganglioma Tumorigenesis, upon Conditional Complex II Loss in Early Sox10+ Cells, *FASEB Bioadv*.
[121] Cirillo, L., Innocenti, S., and Becherucci, F., 2024, Global Epidemiology of Kidney Cancer, *Nephrology Dialysis Transplantation*, **39**(6), pp. 920–928.

[122] Bianchi, M., Sun, M., Jeldres, C., Shariat, S. F., Trinh, Q. D., Briganti, A., Tian, Z., Schmitges, J., Graefen, M., Perrotte, P., Menon, M., Montorsi, F., and Karakiewicz, P. I., 2012, Distribution of Metastatic Sites in Renal Cell Carcinoma: A Population-Based Analysis, *Annals of Oncology*, **23**(4), pp. 973–980.

[123] Rini, B. I., Powles, T., Atkins, M. B., Escudier, B., McDermott, D. F., Suarez, C., Bracarda, S., Stadler, W. M., Donskov, F., Lee, J. L., Hawkins, R., Ravaud, A., Alekseev, B., Staehler, M., Uemura, M., De Giorgi, U., Mellado, B., Porta, C., Melichar, B., Gurney, H., Bedke, J., Choueiri, T. K., Parnis, F., Khaznadar, T., Thobhani, A., Li, S., Piault-Louis, E., Frantz, G., Huseni, M., Schiff, C., Green, M. C., and Motzer, R. J., 2019, Atezolizumab plus Bevacizumab versus Sunitinib in Patients with Previously Untreated Metastatic Renal Cell Carcinoma (IMmotion151): A Multicentre, Open-Label, Phase 3, Randomised Controlled Trial, *The Lancet*, **393**(10189), pp. 2404–2415.

[124] Moch, H., Cubilla, A. L., Humphrey, P. A., Reuter, V. E., and Ulbright, T. M., 2016, The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs—Part A: Renal, Penile, and Testicular Tumours, *Eur Urol*, **70**(1), pp. 93–105.

[125] Fuhrman, S., Lasky, L., and Limas, C., 1982, Prognostic Significance of Morphologic Parameters in Renal Cell Carcinoma, *American Journal of Surgical Pathology*, **6**(7), pp. 655–663.

[126] Chrom, P., Stec, R., Semeniuk-Wojtas, A., Bodnar, L., Spencer, N. J., and Szczylik, C., 2016, Fuhrman Grade and Neutrophil-To-Lymphocyte Ratio Influence on Survival in Patients With Metastatic Renal Cell Carcinoma Treated With First-Line Tyrosine Kinase Inhibitors, *Clin Genitourin Cancer*, **14**(5), pp. 457–464.

[127] Yoo, G. S., Park, W., Pyo, H., Jeong, B. C., Jeon, H. G., Kang, M., Seo, S. Il, Jeon, S. S., Lee, H. M., Choi, H. Y., Park, B. K., Kim, C. K., Park, S. Y., and Kwon, G. Y., 2022, Risk Factors and Patterns of Locoregional Recurrence after Radical Nephrectomy for Locally Advanced Renal Cell Carcinoma, *Cancer Res Treat*, **54**(1), pp. 218–225.

[128] Choi, J., Bang, S., Suh, J., Choi, C. Il, Song, W., Yuk, H. D., Lee, C. H., Kang, M., Choo, S. H., Kim, J. K., Lee, H. H., Jo, J. K., Hwang, E. C., Jeong, C. W., Ko, Y. H., Park, J. Y., Song, C., Seo, S. Il, Chung, J., Kwak, C., and Hong, S. H., 2024, Survival Pattern of Metastatic Renal Cell Carcinoma Patients According to WHO/ISUP Grade: A Long-Term Multi-Institutional Study, *Sci Rep*, **14**(1).

[129] Moch, H., Amin, M. B., Berney, D. M., Compérat, E. M., Gill, A. J., Hartmann, A., Menon, S., Raspollini, M. R., Rubin, M. A., Srigley, J. R., Hoon Tan, P., Tickoo, S. K., Tsuzuki, T., Turajlic, S., Cree, I., and Netto, G. J., 2022, The 2022 World Health Organization Classification of Tumours of the Urinary System and Male Genital Organs—Part A: Renal, Penile, and Testicular Tumours, *Eur Urol*, **82**(5), pp. 458–468.

[130] Gómez-Virgilio, L., Velazquez-Paniagua, M., Cuazozon-Ferrer, L., Silva-Lucero, M. D. C., Gutierrez-Malacara, A. I., Padilla-Mendoza, J. R., Borbolla-Vázquez, J., Díaz-Hernández, J. A., Jiménez-Orozco, F. A., and Cardenas-Aguayo, M. D. C., 2024, Genetics, Pathophysiology, and Current Challenges in Von Hippel–Lindau Disease Therapeutics, *Diagnostics*, **14**(17).

[131] Ghosh, M. C., Zhang, D. L., Ollivierre, H., Eckhaus, M. A., and Rouault, T. A., 2018, Translational Repression of HIF2α Expression in Mice with Chuvash Polycythemia Reverses Polycythemia, *Journal of Clinical Investigation*, **128**(4), pp. 1317–1325.

[132] Clark, P. E., 2009, The Role of VHL in Clear-Cell Renal Cell Carcinoma and Its Relation to Targeted Therapy, *Kidney Int*, **76**(9), pp. 939–945.

[133] Ortiz-Barahona, A., Villar, D., Pescador, N., Amigo, J., and del Peso, L., 2010, Genome-Wide Identification of Hypoxia-Inducible Factor Binding Sites and Target Genes by a Probabilistic Model Integrating Transcription-Profiling Data and in Silico Binding Site Prediction, *Nucleic Acids Res*, **38**(7), pp. 2332–2345.

[134] Smythies, J. A., Sun, M., Masson, N., Salama, R., Simpson, P. D., Murray, E., Neumann, V., Cockman, M. E., Choudhry, H., Ratcliffe, P. J., and Mole, D. R., 2019, Inherent DNA -binding

Specificities of the HIF -1α and HIF -2α Transcription Factors in Chromatin , *EMBO Rep*, **20**(1).
[135] Hoefflin, R., Harlander, S., Schäfer, S., Metzger, P., Kuo, F., Schönenberger, D., Adlesic, M., Peighambari, A., Seidel, P., Chen, C. yi, Consenza-Contreras, M., Jud, A., Lahrmann, B., Grabe, N., Heide, D., Uhl, F. M., Chan, T. A., Duyster, J., Zeiser, R., Schell, C., Heikenwalder, M., Schilling, O., Hakimi, A. A., Boerries, M., and Frew, I. J., 2020, HIF-1α and HIF-2α Differently Regulate Tumour Development and Inflammation of Clear Cell Renal Cell Carcinoma in Mice, *Nat Commun*, **11**(1).
[136] Becker, H. M., 2020, Carbonic Anhydrase IX and Acid Transport in Cancer, *Br J Cancer*, **122**(2), pp. 157–167.
[137] Choueiri, T. K., Cheng, S., Qu, A. Q., Pastorek, J., Atkins, M. B., and Signoretti, S., 2013, Carbonic Anhydrase IX as a Potential Biomarker of Efficacy in Metastatic Clear-Cell Renal Cell Carcinoma Patients Receiving Sorafenib or Placebo: Analysis from the Treatment Approaches in Renal Cancer Global Evaluation Trial (TARGET), *Urologic Oncology: Seminars and Original Investigations*, **31**(8), pp. 1788–1793.
[138] Kierans, S. J., and Taylor, C. T., 2021, Regulation of Glycolysis by the Hypoxia-Inducible Factor (HIF): Implications for Cellular Physiology, *Journal of Physiology*, **599**(1), pp. 23–37.
[139] DepMap, Broad (2024). DepMap 24Q4 Public. Https://Depmap.Org/Portal/.
[140] Beroukhim, R., Brunet, J. P., Di Napoli, A., Mertz, K. D., Seeley, A., Pires, M. M., Linhart, D., Worrell, R. A., Moch, H., Rubin, M. A., Sellers, W. R., Meyerson, M., Linehan, W. M., Kaelin, W. G., and Signoretti, S., 2009, Patterns of Gene Expression and Copy-Number Alterations in von-Hippel Lindau Disease-Associated and Sporadic Clear Cell Carcinoma of the Kidney, *Cancer Res*, **69**(11), pp. 4674–4681.
[141] Abu-Remaileh, M., Persky, N. S., Lee, Y., Root, D. E., and Kaelin, W. G., 2024, Total Loss of VHL Gene Function Impairs Neuroendocrine Cancer Cell Fitness Due to Excessive HIF2α Activity, *Proc Natl Acad Sci U S A*, **121**(40).
[142] Haake, S. M., Weyandt, J. D., and Kimryn Rathmell, W., 2016, Insights into the Genetic Basis of the Renal Cell Carcinomas from the Cancer Genome Atlas, *Molecular Cancer Research*, **14**(7), pp. 589–598.
[143] Turajlic, S., Xu, H., Litchfield, K., Rowan, A., Chambers, T., Lopez, J. I., Nicol, D., O'Brien, T., Larkin, J., Horswell, S., Stares, M., Au, L., Jamal-Hanjani, M., Challacombe, B., Chandra, A., Hazell, S., Eichler-Jonsson, C., Soultati, A., Chowdhury, S., Rudman, S., Lynch, J., Fernando, A., Stamp, G., Nye, E., Jabbar, F., Spain, L., Lall, S., Guarch, R., Falzon, M., Proctor, I., Pickering, L., Gore, M., Watkins, T. B. K., Ward, S., Stewart, A., DiNatale, R., Becerra, M. F., Reznik, E., Hsieh, J. J., Richmond, T. A., Mayhew, G. F., Hill, S. M., McNally, C. D., Jones, C., Rosenbaum, H., Stanislaw, S., Burgess, D. L., Alexander, N. R., and Swanton, C., 2018, Tracking Cancer Evolution Reveals Constrained Routes to Metastases: TRACERx Renal, *Cell*, **173**(3), pp. 581-594.e12.
[144] Mitchell, T. J., Turajlic, S., Rowan, A., Nicol, D., Farmery, J. H. R., O'Brien, T., Martincorena, I., Tarpey, P., Angelopoulos, N., Yates, L. R., Butler, A. P., Raine, K., Stewart, G. D., Challacombe, B., Fernando, A., Lopez, J. I., Hazell, S., Chandra, A., Chowdhury, S., Rudman, S., Soultati, A., Stamp, G., Fotiadis, N., Pickering, L., Au, L., Spain, L., Lynch, J., Stares, M., Teague, J., Maura, F., Wedge, D. C., Horswell, S., Chambers, T., Litchfield, K., Xu, H., Stewart, A., Elaidi, R., Oudard, S., McGranahan, N., Csabai, I., Gore, M., Futreal, P. A., Larkin, J., Lynch, A. G., Szallasi, Z., Swanton, C., and Campbell, P. J., 2018, Timing the Landmark Events in the Evolution of Clear Cell Renal Cell Cancer: TRACERx Renal, *Cell*, **173**(3), pp. 611-623.e17.
[145] Harlander, S., Schönenberger, D., Toussaint, N. C., Prummer, M., Catalano, A., Brandt, L., Moch, H., Wild, P. J., and Frew, I. J., 2017, Combined Mutation in Vhl, Trp53 and Rb1 Causes Clear Cell Renal Cell Carcinoma in Mice, *Nat Med*, **23**(7), pp. 869–877.
[146] Kleymenova, E., Everitt, J. I., Pluta, L., Portis, M., Gnarra, J. R., and Walker, C. L., 2004, Susceptibility to Vascular Neoplasms but No Increased Susceptibility to Renal Carcinogenesis in Vhl Knockout Mice, *Carcinogenesis*, **25**(3), pp. 309–315.
[147] Motzer, R. J., Escudier, B., McDermott, D. F., George, S., Hammers, H. J., Srinivas, S., Tykodi, S. S., Sosman, J. A., Procopio, G., Plimack, E. R., Castellano, D., Choueiri, T. K., Gurney, H., Donskov, F.,

Bono, P., Wagstaff, J., Gauler, T. C., Ueda, T., Tomita, Y., Schutz, F. A., Kollmannsberger, C., Larkin, J., Ravaud, A., Simon, J. S., Xu, L.-A., Waxman, I. M., and Sharma, P., 2015, Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma, *New England Journal of Medicine*, **373**(19), pp. 1803–1813.

[148] Xu, R., Wang, K., Rizzi, J. P., Huang, H., Grina, J. A., Schlachter, S. T., Wang, B., Wehn, P. M., Yang, H., Dixon, D. D., Czerwinski, R. M., Du, X., Ged, E. L., Han, G., Tan, H., Wong, T., Xie, S., Josey, J. A., and Wallace, E. M., 2019, 3-[(1 S,2 S,3 R)-2,3-Difluoro-1-Hydroxy-7-Methylsulfonylindan-4-Yl]Oxy-5-Fluorobenzonitrile (PT2977), a Hypoxia-Inducible Factor 2α (HIF-2α) Inhibitor for the Treatment of Clear Cell Renal Cell Carcinoma, *J Med Chem*, **62**(15), pp. 6876–6893.

[149] Amendolare, A., Marzano, F., Petruzzella, V., Vacca, R. A., Guerrini, L., Pesole, G., Sbisà, E., and Tullo, A., 2022, The Underestimated Role of the P53 Pathway in Renal Cancer, *Cancers (Basel)*, **14**(23).

[150] Rhoades Smith, K. E., and Bilen, M. A., 2019, A Review of Papillary Renal Cell Carcinoma and MET Inhibitors, *Kidney Cancer*, **3**(3), pp. 151–161.

[151] Pan, H., Ye, L., Zhu, Q., Yang, Z., and Hu, M., 2020, The Effect of the Papillary Renal Cell Carcinoma Subtype on Oncological Outcomes, *Sci Rep*, **10**(1).

[152] Volpe, A., Novara, G., Antonelli, A., Bertini, R., Billia, M., Carmignani, G., Cosciani Cunico, S., Longo, N., Martignoni, G., Minervini, A., Mirone, V., Simonato, A., Terrone, C., Zattoni, F., Ficarra, V., De Cobelli, O., Martorana, G., Schiavina, R., Corti, S., Simeone, C., Castelli, M., Cimino, S., Favilla, V., Morgia, G., Imbimbo, C., Carini, M., Masieri, L., Serni, S., Oneto, F., Varca, V., Rocco, F., Valotto, C., Costantini, E., Porena, M., Zucchi, A., Ciciliato, S., Lampropoulou, N., Siracusano, S., Fontana, D., Gontero, P., Tizzani, A., Artibani, W., Brunelli, M., Montorsi, F., Petralia, G., Roscigno, M., and Strada, E., 2012, Chromophobe Renal Cell Carcinoma (RCC): Oncological Outcomes and Prognostic Factors in a Large Multicentre Series, *BJU Int*, **110**(1), pp. 76–83.

[153] Davis, C. F., Ricketts, C. J., Wang, M., Yang, L., Sofia, H. J., et al., 2014, The Somatic Genomic Landscape of Chromophobe Renal Cell Carcinoma, *Cancer Cell*, **26**(3), pp. 319–330.

[154] Durinck, S., Stawiski, E. W., Pavía-Jiménez, A., Modrusan, Z., Kapur, P., Jaiswal, B. S., Zhang, N., Toffessi-Tcheuyap, V., Nguyen, T. T., Pahuja, K. B., Chen, Y. J., Saleem, S., Chaudhuri, S., Heldens, S., Jackson, M., Peña-Llopis, S., Guillory, J., Toy, K., Ha, C., Harris, C. J., Holloman, E., Hill, H. M., Stinson, J., Rivers, C. S., Janakiraman, V., Wang, W., Kinch, L. N., Grishin, N. V., Haverty, P. M., Chow, B., Gehring, J. S., Reeder, J., Pau, G., Wu, T. D., Margulis, V., Lotan, Y., Sagalowsky, A., Pedrosa, I., De Sauvage, F. J., Brugarolas, J., and Seshagiri, S., 2015, Spectrum of Diverse Genomic Alterations Define Non-Clear Cell Renal Carcinoma Subtypes, *Nat Genet*, **47**(1), pp. 13–21.

[155] Gill, A. J., Hes, O., Papathomas, T., Edivcova´,Edivcova´, Msc, M. S. ˇ, Tan, P. H., Agaimy, A., Per, #, Andresen, A., Kedziora, A., Clarkson, A., Toon, C. W., Sioson, L., Watson, N., Chou, A., Paik, J., Clifton-Bligh, R. J., Robinson, B. G., Benn, D. E., Hills, K., Maclean, F., Niemeijer, N. D., Vlatkovic, L., Hartmann, A., Corssmit, E. P. M., Van Leenders, G. J. L. H., Przybycin, C., Mckenney, J. K., Magi-Galluzzi, C., Yilmaz, A., Yu, D., Nicoll, K. D., Yong, J. L., Sibony, M., Yakirevich, E., Fleming, S., Chow, C. W., Miettinen, M., Michal, M., and Trpkov, K., 2014, *Succinate Dehydrogenase (SDH)-Deficient Renal Carcinoma: A Morphologically Distinct Entity A Clinicopathologic Series of 36 Tumors From 27 Patients*. [Online]. Available: http://links.lww.com/PAS/A224.

[156] Gill, A. J., 2018, Succinate Dehydrogenase (SDH)-Deficient Neoplasia, *Histopathology*, **72**(1), pp. 106–116.

[157] Cardaci, S., Zheng, L., Mackay, G., Van Den Broek, N. J. F., Mackenzie, E. D., Nixon, C., Stevenson, D., Tumanov, S., Bulusu, V., Kamphorst, J. J., Vazquez, A., Fleming, S., Schiavi, F., Kalna, G., Blyth, K., Strathdee, D., and Gottlieb, E., 2015, Pyruvate Carboxylation Enables Growth of SDH-Deficient Cells by Supporting Aspartate Biosynthesis, *Nat Cell Biol*, **17**(10), pp.

1317–1326.
[158] Lussey-Lepoutre, C., Hollinshead, K. E. R., Ludwig, C., Menara, M., Morin, A., Castro-Vega, L. J., Parker, S. J., Janin, M., Martinelli, C., Ottolenghi, C., Metallo, C., Gimenez-Roqueplo, A. P., Favier, J., and Tennant, D. A., 2015, Loss of Succinate Dehydrogenase Activity Results in Dependency on Pyruvate Carboxylation for Cellular Anabolism, *Nat Commun*, **6**.
[159] Ariaans, G., Tiersma, J. F., Evers, B., Gerding, A., Waaijer, S. J. H., Koster, R. A., Touw, D. J., Bakker, B. M., Reijngoud, D. J., de Jong, S., and Jalving, M., 2024, Everolimus Decreases [U-13C]Glucose Utilization by Pyruvate Carboxylase in Breast Cancer Cells in Vitro and in Vivo, *Biomedicine and Pharmacotherapy*, **173**.
[160] Altman, B. J., Stine, Z. E., and Dang, C. V., 2016, From Krebs to Clinic: Glutamine Metabolism to Cancer Therapy, *Nat Rev Cancer*, **16**(10), pp. 619–634.
[161] Fendt, S. M., Bell, E. L., Keibler, M. A., Olenchock, B. A., Mayers, J. R., Wasylenko, T. M., Vokes, N. I., Guarente, L., Heiden, M. G. V., and Stephanopoulos, G., 2013, Reductive Glutamine Metabolism Is a Function of the α-Ketoglutarate to Citrate Ratio in Cells, *Nat Commun*, **4**.
[162] Deberardinis, R. J., Mancuso, A., Daikhin, E., Nissim, I., Yudkoff, M., Wehrli, S., and Thompson, C. B., 2007, *Beyond Aerobic Glycolysis: Transformed Cells Can Engage in Glutamine Metabolism That Exceeds the Requirement for Protein and Nucleotide Synthesis*. [Online]. Available: www.pnas.org/cgi/content/full/.
[163] Ricci, L., Stanley, F. U., Eberhart, T., Mainini, F., Sumpton, D., and Cardaci, S., 2023, Pyruvate Transamination and NAD Biosynthesis Enable Proliferation of Succinate Dehydrogenase-Deficient Cells by Supporting Aerobic Glycolysis, *Cell Death Dis*, **14**(7).
[164] Kim, M., Gwak, J., Hwang, S., Yang, S., and Jeong, S. M., 2019, Mitochondrial GPT2 Plays a Pivotal Role in Metabolic Adaptation to the Perturbation of Mitochondrial Glutamine Metabolism, *Oncogene*, **38**(24), pp. 4729–4738.
[165] Wang, Z. M., Ying, Z., Bosy-Westphal, A., Zhang, J., Schautz, B., Later, W., Heymsfield, S. B., and Müller, M. J., 2010, Specific Metabolic Rates of Major Organs and Tissues across Adulthood: Evaluation by Mechanistic Model of Resting Energy Expenditure, *American Journal of Clinical Nutrition*, **92**(6), pp. 1369–1377.
[166] Bhargava, P., and Schnellmann, R. G., 2017, Mitochondrial Energetics in the Kidney, *Nat Rev Nephrol*, **13**(10), pp. 629–646.
[167] Cargill, K., Hemker, S. L., Clugston, A., Murali, A., Mukherjee, E., Liu, J., Bushnell, D., Bodnar, A. J., Saifudeen, Z., Ho, J., Bates, C. M., Kostka, D., Goetzman, E. S., and Sims-Lucas, S., 2019, Von Hippel-Lindau Acts as a Metabolic Switch Controlling Nephron Progenitor Differentiation, *Journal of the American Society of Nephrology*, **30**(7), pp. 1192–1205.
[168] Coffey, N. J., and Simon, M. C., 2024, Metabolic Alterations in Hereditary and Sporadic Renal Cell Carcinoma, *Nat Rev Nephrol*, **20**(4), pp. 233–250.
[169] Zhu, H., Wang, X., Lu, S., and Ou, K., 2023, Metabolic Reprogramming of Clear Cell Renal Cell Carcinoma, *Front Endocrinol (Lausanne)*, **14**.
[170] Chakraborty, S., Balan, M., Sabarwal, A., Choueiri, T. K., and Pal, S., 2021, Metabolic Reprogramming in Renal Cancer: Events of a Metabolic Disease, *Biochim Biophys Acta Rev Cancer*, **1876**(1).
[171] Abbott, K. L., Ali, A., Reinfeld, B. I., Deik, A., Subudhi, S., Landis, M. D., Hongo, R. A., Young, K. L., Kunchok, T., Nabel, C. S., Crowder, K. D., Kent, J. R., Madariaga, M. L. L., Jain, R. K., Beckermann, K. E., Lewis, C. A., Clish, C. B., Muir, A., Rathmell, W. K., Rathmell, J., and Heiden, M. G. V., 2024, Metabolite Profiling of Human Renal Cell Carcinoma Reveals Tissue-Origin Dominance in Nutrient Availability, *Elife*, **13**.
[172] Aggarwal, R. K., Luchtel, R. A., Machha, V., Tischer, A., Zou, Y., Pradhan, K., Ashai, N., Ramachandra, N., Albanese, J. M., Yang, J., Wang, X., Aluri, S., Gordon, S., Aboumohamed, A., Gartrell, B. A., Hafizi, S., Pullman, J., and Shenoy, N., 2021, Functional Succinate Dehydrogenase Deficiency Is a Common Adverse Feature of Clear Cell Renal Cancer MEDICAL SCIENCES, **118**, p. 2106947118.

[173] Carvalho, L. B., Jorge, S., López-Fernández, H., Lodeiro, C., Dhir, R., Campos Pinheiro, L., Medeiros, M., Santos, H. M., and Capelo, J. L., 2023, Proteomic Analysis of Chromophobe Renal Cell Carcinoma and Benign Renal Oncocytoma Biopsies Reveals Shared Metabolic Dysregulation, *Clin Proteomics*, **20**(1).
[174] Xiao, Y., Clima, R., Busch, J., Rabien, A., Kilic, E., Villegas, S. L., Timmermann, B., Attimonelli, M., Jung, K., and Meierhofer, D., 2020, Decreased Mitochondrial DNA Content Drives OXPHOS Dysregulation in Chromophobe Renal Cell Carcinoma, *Cancer Res*, **80**(18), pp. 3830–3840.
[175] Reznik, E., Wang, Q., and La, K., 2017, Mitochondrial Respiratory Gene Is Suppressed in Many Cancers.
[176] Zong, W. X., Rabinowitz, J. D., and White, E., 2016, Mitochondria and Cancer, *Mol Cell*, **61**(5), pp. 667–676.
[177] Kukat, C., Davies, K. M., Wurm, C. A., Spåhr, H., Bonekamp, N. A., Kühl, I., Joos, F., Polosa, P. L., Park, C. B., Posse, V., Falkenberg, M., Jakobs, S., Kühlbrandt, W., and Larsson, N. G., 2015, Cross-Strand Binding of TFAM to a Single MtDNA Molecule Forms the Mitochondrial Nucleoid, *Proc Natl Acad Sci U S A*, **112**(36), pp. 11288–11293.
[178] LaGory, E. L., Wu, C., Taniguchi, C. M., Ding, C. K. C., Chi, J. T., von Eyben, R., Scott, D. A., Richardson, A. D., and Giaccia, A. J., 2015, Suppression of PGC-1α Is Critical for Reprogramming Oxidative Metabolism in Renal Cell Carcinoma, *Cell Rep*, **12**(1), pp. 116–127.
[179] Li, S., Li, W., Yuan, J., Bullova, P., Wu, J., Zhang, X., Liu, Y., Plescher, M., Rodriguez, J., Bedoya-Reina, O. C., Jannig, P. R., Valente-Silva, P., Yu, M., Henriksson, M. A., Zubarev, R. A., Smed-Sörensen, A., Suzuki, C. K., Ruas, J. L., Holmberg, J., Larsson, C., Christofer Juhlin, C., von Kriegsheim, A., Cao, Y., and Schlisio, S., 2022, Impaired Oxygen-Sensitive Regulation of Mitochondrial Biogenesis within the von Hippel–Lindau Syndrome, *Nat Metab*, **4**(6), pp. 739–758.
[180] Krencz, I., Vetlényi, E., Dankó, T., Petővári, G., Moldvai, D., Sztankovics, D., Raffay, R., Mészáros, K., Sebestyén, E., Végső, G., Pápay, J., and Sebestyén, A., 2022, Metabolic Adaptation as Potential Target in Papillary Renal Cell Carcinomas Based on Their In Situ Metabolic Characteristics, *Int J Mol Sci*, **23**(18).
[181] Kubala, J. M., Laursen, K. B., Schreiner, R., Williams, R. M., van der Mijn, J. C., Crowley, M. J., Mongan, N. P., Nanus, D. M., Heller, D. A., and Gudas, L. J., 2023, NDUFA4L2 Reduces Mitochondrial Respiration Resulting in Defective Lysosomal Trafficking in Clear Cell Renal Cell Carcinoma, *Cancer Biol Ther*, **24**(1).
[182] Liu, L., Lan, G., Peng, L., Xie, X., Peng, F., Yu, S., Wang, Y., and Tang, X., 2016, NDUFA4L2 Expression Predicts Poor Prognosis in Clear Cell Renal Cell Carcinoma Patients, *Ren Fail*, **38**(8), pp. 1199–1205.
[183] Tello, D., Balsa, E., Acosta-Iborra, B., Fuertes-Yebra, E., Elorza, A., Ordóñez, Á., Corral-Escariz, M., Soro, I., López-Bernardo, E., Perales-Clemente, E., Martínez-Ruiz, A., Enríquez, J. A., Aragonés, J., Cadenas, S., and Landázuri, M. O., 2011, Induction of the Mitochondrial NDUFA4L2 Protein by HIF-1α Decreases Oxygen Consumption by Inhibiting Complex i Activity, *Cell Metab*, **14**(6), pp. 768–779.
[184] Čunátová, K., Vrbacký, M., Puertas-Frias, G., Alán, L., Vanišová, M., Saucedo-Rodríguez, M. J., Houštěk, J., Fernández-Vizarra, E., Neužil, J., Pecinová, A., Pecina, P., and Mráček, T., 2024, Mitochondrial Translation Is the Primary Determinant of Secondary Mitochondrial Complex I Deficiencies, *iScience*, p. 110560.
[185] Williamson, S. R., Eble, J. N., Amin, M. B., Gupta, N. S., Smith, S. C., Sholl, L. M., Montironi, R., Hirsch, M. S., and Hornick, J. L., 2015, Succinate Dehydrogenase-Deficient Renal Cell Carcinoma: Detailed Characterization of 11 Tumors Defining a Unique Subtype of Renal Cell Carcinoma, *Modern Pathology*, **28**(1), pp. 80–94.
[186] Housley, S. L., Lindsay, R. S., Young, B., McConachie, M., Mechan, D., Baty, D., Christie, L., Rahilly, M., Qureshi, K., and Fleming, S., 2010, Renal Carcinoma with Giant Mitochondria

Associated with Germ-Line Mutation and Somatic Loss of the Succinate Dehydrogenase B Gene, *Histopathology*, **56**(3), pp. 405–408.
[187] Kuroda, N., Yorita, K., Nagasaki, M., Harada, Y., Ohe, C., Jeruc, J., Raspollini, M. R., Michal, M., Hes, O., and Amin, M. B., 2016, Review of Succinate Dehydrogenase-Deficient Renal Cell Carcinoma with Focus on Clinical and Pathobiological Aspects, *Polish Journal of Pathology*, **67**(1), pp. 3–7.
[188] Bezwada, D., Perelli, L., Lesner, N. P., Cai, L., Brooks, B., Wu, Z., Vu, H. S., Sondhi, V., Cassidy, D. L., Kasitinon, S., Kelekar, S., Cai, F., Aurora, A. B., Patrick, M., Leach, A., Ghandour, R., Zhang, Y., Do, D., McDaniel, P., Sudderth, J., Dumesnil, D., House, S., Rosales, T., Poole, A. M., Lotan, Y., Woldu, S., Bagrodia, A., Meng, X., Cadeddu, J. A., Mishra, P., Garcia-Bermudez, J., Pedrosa, I., Kapur, P., Courtney, K. D., Malloy, C. R., Genovese, G., Margulis, V., and DeBerardinis, R. J., 2024, Mitochondrial Complex I Promotes Kidney Cancer Metastasis, *Nature*.
[189] Cornejo, K. M., Lu, M., Yang, P., Wu, S., Cai, C., Zhong, W. De, Olumi, A., Young, R. H., and Wu, C. L., 2015, Succinate Dehydrogenase B: A New Prognostic Biomarker in Clear Cell Renal Cell Carcinoma, *Hum Pathol*, **46**(6), pp. 820–826.
[190] Neves, J. B., Roberts, K., Nguyen, J. S., El Sheikh, S., Tran-Dang, M. A., Horsfield, C., Mumtaz, F., Campbell, P., Stauss, H., Tran, M. G. B., and Mitchell, T., 2022, Defining the Origin, Evolution, and Immune Composition of SDH-Deficient Renal Cell Carcinoma, *iScience*, **25**(11).
[191] Ricketts, C. J., Killian, J. K., Vocke, C. D., Wang, Y., Merino, M. J., Meltzer, P. S., and Linehan, W. M., 2022, Kidney Tumors Associated with Germline Mutations of FH and SDHB Show a CpG Island Methylator Phenotype (CIMP), *PLoS One*, **17**(12 December).
[192] Piantadosi, C. A., and Suliman, H. B., 2008, Transcriptional Regulation of SDHa Flavoprotein by Nuclear Respiratory Factor-1 Prevents Pseudo-Hypoxia in Aerobic Cardiac Cells, *Journal of Biological Chemistry*, **283**(16), pp. 10967–10977.
[193] Fang, Z., Sun, Q., Yang, H., and Zheng, J., 2021, SDHB Suppresses the Tumorigenesis and Development of CcRCC by Inhibiting Glycolysis, *Front Oncol*, **11**.
[194] Yang, J., Zhou, Y., Li, Y., Hu, W., Yuan, C., Chen, S., Ye, G., Chen, Y., Wu, Y., Liu, J., Wang, Y., Du, J., and Tong, X., 2022, Functional Deficiency of Succinate Dehydrogenase Promotes Tumorigenesis and Development of Clear Cell Renal Cell Carcinoma through Weakening of Ferroptosis, *Bioengineered*, **13**(4), pp. 11187–11207.
[195] Yang, G., Cheng, J., Xu, J., Shen, C., Lu, X., He, C., Huang, J., He, M., Cheng, J., and Wang, H., 2024, Metabolic Heterogeneity in Clear Cell Renal Cell Carcinoma Revealed by Single-Cell RNA Sequencing and Spatial Transcriptomics, *J Transl Med*, **22**(1).
[196] Meierhofer, D., Mayr, J. A., Foetschl, U., Berger, A., Fink, K., Schmeller, N., Hacker, G. W., Hauser-Kronberger, C., Kofler, B., and Sperl, W., 2004, Decrease of Mitochondrial DNA Content and Energy Metabolism in Renal Cell Carcinoma, *Carcinogenesis*, **25**(6), pp. 1005–1010.
[197] Tian, J., Luo, J., Zeng, X., Ke, C., Wang, Y., Liu, Z., Li, L., Zhang, Y., Hu, Z., and Yang, C., 2024, Targeting Oxidative Phosphorylation to Increase the Efficacy of Immune-Combination Therapy in Renal Cell Carcinoma, *J Immunother Cancer*, **12**(2), p. e008226.
[198] Lebleu, V. S., O'Connell, J. T., Gonzalez Herrera, K. N., Wikman, H., Pantel, K., Haigis, M. C., De Carvalho, F. M., Damascena, A., Domingos Chinen, L. T., Rocha, R. M., Asara, J. M., and Kalluri, R., 2014, PGC-1α Mediates Mitochondrial Biogenesis and Oxidative Phosphorylation in Cancer Cells to Promote Metastasis, *Nat Cell Biol*, **16**(10), pp. 992–1003.
[199] Martínez-Jiménez, F., Movasati, A., Brunner, S. R., Nguyen, L., Priestley, P., Cuppen, E., and Van Hoeck, A., 2023, Pan-Cancer Whole-Genome Comparison of Primary and Metastatic Solid Tumours, *Nature*, **618**(7964), pp. 333–341.
[200] Takeda, K., Bastacky, S., Dhir, R., Mohebnasab, M., and Quiroga-Garza, G. M., 2024, Morphological Characteristics of SETD2-Mutated Locally Advanced Clear Cell Renal Cell Carcinoma: Comparison with BAP1-Mutated Clear Cell Renal Cell Carcinoma, *Ann Diagn Pathol*, **68**.
[201] Liu, J., Hanavan, P. D., Kras, K., Ruiz, Y. W., Castle, E. P., Lake, D. F., Chen, X., O'Brien, D., Luo, H., Robertson, K. D., Gu, H., and Ho, T. H., 2019, Loss of SETD2 Induces a Metabolic Switch in Renal Cell Carcinoma Cell Lines toward Enhanced Oxidative Phosphorylation, *J Proteome Res*, **18**(1),

pp. 331–340.
[202] Papathomas, T. G., Gaal, J., Corssmit, E. P. M., Oudijk, L., Korpershoek, E., Heimdal, K., Bayley, J. P., Morreau, H., Van Dooren, M., Papaspyrou, K., Schreiner, T., Hansen, T., Andresen, P. A., Restuccia, D. F., Van Kessel, I., Van Leenders, G. J. L. H., Kros, J. M., Looijenga, L. H. J., Hofland, L. J., Mann, W., Van Nederveen, F. H., Mete, O., Asa, S. L., De Krijger, R. R., and Dinjens, W. N. M., 2014, Non-Pheochromocytoma (PCC)/Paraganglioma (PGL) Tumors in Patients with Succinate Dehydrogenase-Related PCC-PGL Syndromes: A Clinicopathological and Molecular Analysis, *Eur J Endocrinol*, **170**(1), pp. 1–12.
[203] Miklovicova, S., Volpini, L., Sanovec, O., Monaco, F., Vanova, K. H., Novak, J., Boukalova, S., Zobalova, R., Klezl, P., Tomasetti, M., Bobek, V., Fiala, V., Vcelak, J., Santarelli, L., Bielcikova, Z., Komrskova, K., Kolostova, K., Pacak, K., Dvorakova, S., and Neuzil, J., 2025, Mitochondrial Respiratory Complex II Is Altered in Renal Carcinoma, *Biochim Biophys Acta Mol Basis Dis*, **1871**(1).
[204] Lemarie, A., Huc, L., Pazarentzos, E., Mahul-Mellier, A. L., and Grimm, S., 2011, Specific Disintegration of Complex II Succinate:Ubiquinone Oxidoreductase Links PH Changes to Oxidative Stress for Apoptosis Induction, *Cell Death Differ*, **18**(2), pp. 338–349.
[205] Reynolds, M. B., Hong, H. S., Michmerhuizen, B. C., Lawrence, A.-L. E., Zhang, L., Knight, J. S., Lyssiotis, C. A., Abuaita, B. H., and O'riordan, M. X., 2023, *Cardiolipin Coordinates Inflammatory Metabolic Reprogramming through Regulation of Complex II Disassembly and Degradation*. [Online]. Available: https://www.science.org.
[206] Sharma, P., Maklashina, E., Voehler, M., Balintova, S., Dvorakova, S., Kraus, M., Vanova, K. H., Nahacka, Z., Zobalova, R., Boukalova, S., Cunatova, K., Mracek, T., Ghayee, H. K., Pacak, K., Rohlena, J., Neuzil, J., Cecchini, G., and Iverson, T. M., 2024, Disordered-to-Ordered Transitions in Assembly Factors Allow the Complex II Catalytic Subunit to Switch Binding Partners, *Nat Commun*, **15**(1).
[207] Maklashina, E., Rajagukguk, S., Iverson, T. M., and Cecchini, G., 2018, The Unassembled Flavoprotein Subunits of Human and Bacterial Complex II Have Impaired Catalytic Activity and Generate Only Minor Amounts of ROS, *Journal of Biological Chemistry*, **293**(20), pp. 7754–7765.
[208] Ashkenazi, A., Fairbrother, W. J., Leverson, J. D., and Souers, A. J., 2017, From Basic Apoptosis Discoveries to Advanced Selective BCL-2 Family Inhibitors, *Nat Rev Drug Discov*, **16**(4), pp. 273–284.
[209] Neuzil, J., Dong, L. F., Rohlena, J., Truksa, J., and Ralph, S. J., 2013, Classification of Mitocans, Anti-Cancer Drugs Acting on Mitochondria, *Mitochondrion*, **13**(3), pp. 199–208.
[210] Rohlenova, K., Sachaphibulkij, K., Stursa, J., Bezawork-Geleta, A., Blecha, J., Endaya, B., Werner, L., Cerny, J., Zobalova, R., Goodwin, J., Spacek, T., Alizadeh Pesdar, E., Yan, B., Nguyen, M. N., Vondrusova, M., Sobol, M., Jezek, P., Hozak, P., Truksa, J., Rohlena, J., Dong, L. F., and Neuzil, J., 2017, Selective Disruption of Respiratory Supercomplexes as a New Strategy to Suppress Her2high Breast Cancer, *Antioxid Redox Signal*, **26**(2), pp. 84–103.
[211] Bielcikova, Z., Stursa, J., Krizova, L., Dong, L., Spacek, J., Hlousek, S., Vocka, M., Rohlenova, K., Bartosova, O., Cerny, V., Padrta, T., Pesta, M., Michalek, P., Hubackova, S. S., Kolostova, K., Pospisilova, E., Bobek, V., Klezl, P., Zobalova, R., Endaya, B., Rohlena, J., Petruzelka, L., Werner, L., and Neuzil, J., 2023, Mitochondrially Targeted Tamoxifen in Patients with Metastatic Solid Tumours: An Open-Label, Phase I/Ib Single-Centre Trial, *EClinicalMedicine*, **57**.
[212] Bielcikova, Z., Werner, L., Stursa, J., Cerny, V., Krizova, L., Spacek, J., Hlousek, S., Vocka, M., Bartosova, O., Pesta, M., Kolostova, K., Klezl, P., Bobek, V., Truksa, J., Stemberkova-Hubackova, S., Petruzelka, L., Michalek, P., and Neuzil, J., 2023, Mitochondrially Targeted Tamoxifen as Anticancer Therapy: Case Series of Patients with Renal Cell Carcinoma Treated in a Phase I/Ib Clinical Trial, *Ther Adv Med Oncol*, **15**.
[213] Hart, M. L., Quon, E., Vigil, A. L. B. G., Engstrom, I. A., Newsom, O. J., Davidsen, K.,

Hoellerbauer, P., Carlisle, S. M., and Sullivan, L. B., 2023, Mitochondrial Redox Adaptations Enable Alternative Aspartate Synthesis in SDH-Deficient Cells, *Elife*, **12**.

[214] Saxena, N., Maio, N., Crooks, D. R., Ricketts, C. J., Yang, Y., Wei, M. H., Fan, T. W. M., Lane, A. N., Sourbier, C., Singh, A., Killian, J. K., Meltzer, P. S., Vocke, C. D., Rouault, T. A., and Linehan, W. M., 2016, SDHB-Deficient Cancers: The Role of Mutations That Impair Iron Sulfur Cluster Delivery, *J Natl Cancer Inst*, **108**(1).

[215] Lorendeau, D., Rinaldi, G., Boon, R., Spincemaille, P., Metzger, K., Jäger, C., Christen, S., Dong, X., Kuenen, S., Voordeckers, K., Verstreken, P., Cassiman, D., Vermeersch, P., Verfaillie, C., Hiller, K., and Fendt, S. M., 2017, Dual Loss of Succinate Dehydrogenase (SDH) and Complex I Activity Is Necessary to Recapitulate the Metabolic Phenotype of SDH Mutant Tumors, *Metab Eng*, **43**, pp. 187–197.

[216] Grønborg, S., Darin, N., Miranda, M. J., Damgaard, B., Cayuela, J. A., Oldfors, A., Kollberg, G., Hansen, T. V. O., Ravn, K., Wibrand, F., and Østergaard, E., 2017, Leukoencephalopathy Due to Complex II Deficiency and Bi-Allelic SDHB Mutations: Further Cases and Implications for Genetic Counselling, *JIMD Reports*, *Springer*, pp. 69–77.

[217] Renkema, G. H., Wortmann, S. B., Smeets, R. J., Venselaar, H., Antoine, M., Visser, G., Ben-Omran, T., Van Den Heuvel, L. P., Timmers, H. J., Smeitink, J. A., and Rodenburg, R. J. T., 2015, SDHA Mutations Causing a Multisystem Mitochondrial Disease: Novel Mutations and Genetic Overlap with Hereditary Tumors, *European Journal of Human Genetics*, **23**(2), pp. 202–209.

[218] Alston, C. L., Davison, J. E., Meloni, F., van der Westhuizen, F. H., He, L., Hornig-Do, H. T., Peet, A. C., Gissen, P., Goffrini, P., Ferrero, I., Wassmer, E., McFarland, R., and Taylor, R. W., 2012, Recessive Germline SDHA and SDHB Mutations Causing Leukodystrophy and Isolated Mitochondrial Complex II Deficiency, *J Med Genet*, **49**(9), pp. 569–577.

[219] Kľučková, K., Thakker, A., Vettore, L., Escribano-Gonzalez, C., Hindshaw, R. L., Tearle, J. L. E., Goncalves, J., Kaul, B., Lavery, G. G., Favier, J., and Tennant, D. A., 2020, Succinate Dehydrogenase Deficiency in a Chromaffin Cell Model Retains Metabolic Fitness through the Maintenance of Mitochondrial Nadh Oxidoreductase Function, *FASEB Journal*, **34**(1), pp. 303–315.

[220] Maio, N., Kim, K. S., Singh, A., and Rouault, T. A., 2017, A Single Adaptable Cochaperone-Scaffold Complex Delivers Nascent Iron-Sulfur Clusters to Mammalian Respiratory Chain Complexes I–III, *Cell Metab*, **25**(4), pp. 945-953.e6.