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Pavel Jakoubě

Mitochondriální tumor supresory

Mitochondrial tumour suppressors

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Advisor: Mgr. Zuzana Kečkéšová, Ph.D.

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Acknowledgements

Abstract

Cancer is one of the most feared diseases in our modern society and many resources are spent on developing new ways of diagnosis, prevention and treatment. Luckily for us, our bodies already have a first line of defence against carcinogenesis – proteins called tumour suppressors. Studying these proteins can give us an important insight into the inner workings of this disease and can also show us new ways of combating it. One of the signs of cancer cells is dysregulation of metabolic processes and since mitochondria play a pivotal part in many of these processes, we wanted to research the identity and role of mitochondrial tumour suppressors. Indeed, several such tumour suppressors have been identified, having a plethora of functions such as modulating the activity of other mitochondrial enzymes, directly participating in cellular metabolic pathways, affecting reactive oxygen species production and modulating hypoxia-induced signalling. The focus of this work is to gather the available information about these important protective proteins.

Key words: cancer, tumour suppressor, SIRT3, SIRT4, POX/PRODH, MTUS1/MTSG1, FUS1/TUSC2, LACTB, FH, SDH, mitochondrial

Abstrakt

Rakovina zůstává jedním z nejobávanějších onemocnění naší doby a do vývoje nových způsobů diagnostiky, prevence a léčby je investováno nemalé množství finančních prostředků. Naštěstí máme v těle i vlastní obranné mechanismy, které brání nádorové transformaci – takzvané tumor supresorové proteiny. Studium těchto proteinů nám může nabídnout nejen vhled do vnitřních procesů této nemoci, ale také odhalit nové způsoby, jak s ní bojovat. Nápadným znakem rakoviny je deregulace různých metabolických procesů, a jelikož mitochondrie hrají stěžejní roli v mnoha těchto procesech, zajímalo nás, zda se v mitochondrii tumor supresory nachází a jaká je jejich role. Několik mitochondriálních tumor supresorů již bylo identifikováno a bylo zjištěno, že zastávají různé funkce (např. úprava aktivity ostatních enzymů či zánětlivé odpovědi, přímý podíl v různých metabolických drahách, ovlivňování produkce volných radikálů a HIF signalizace). Cílem této práce je shrnout dostupné informace o těchto důležitých proteinech.

Klíčová slova: rakovina, tumor supresory, SIRT3, SIRT4, POX/PRODH, MTUS1/MTSG1, FUS1/TUSC2, LACTB, FH, SDH, mitochondriální

List of Abbreviations

AA	Amino acid	**NF-κB**	Nuclear factor kappa B
AMPK	AMP-activated protein kinase	**NFAT**	Nuclear factor of activated T-cells
ATIP	Angiotensin-II type 2 receptor-interacting protein	**PIG**	p53 induced gene
CRC	Colorectal cancer	**PPP**	Pentose phosphate pathway
CDC	Cyclin-dependent kinase	**PPAR**	Peroxisome proliferator-activated receptor
COX	Cyclooxygenase	**PTEN**	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase
DOX	Doxycycline	**PI3K**	Phosphoinositide 3-kinase
ETC	Electron transport chain	**PRODH**	Proline dehydrogenase
EGFR	Epidermal growth factor receptor	**POX**	Proline oxidase
EMT	Epithelial-mesenchymal transition	**PGE2**	Prostaglandin E2
FH	Fumarate hydratase	**PYCR**	Pyrroline-5-carboxylate reductase
FUS1	Fusion protein 1	**PDH**	Pyruvate dehydrogenase
GC	Gastric cancer	**ROS**	Reactive oxygen species
GDH	Glutamate dehydrogenase	**RCC**	renal cell carcinoma
GFP	Green fluorescent protein	**LACTB**	Serine beta-lactamase-like protein
HCC	Hepatocellular carcinoma	**SDH**	Succinate dehydrogenase
HIF	Hypoxia inducible factor	**SOD**	Superoxide dismutase
IRP	Iron regulatory protein	**TRAIL**	TNF-related apoptosis-inducing ligand
IRE	Iron response element	**TfR**	Transferrin receptor
KO	Knockout	**TCA**	Tricarboxylic acid
mTOR	Mammalian target of rapamycin	**TNF**	Tumour necrosis factor
MMP	Matrix metalloproteinase	**TUSC2**	Tumour suppressor candidate 2
MTUS1	Microtubule-associated scaffold protein 1	**WT**	Wild type
MTSG1	Mitochondrial tumour suppressor 1	**P5C**	Δ^1-pyrroline-5-carboxylate
MEF	Mouse embryonic fibroblasts		

Contents

1   Introduction

Throughout 2018 18.1 million people were diagnosed with cancer worldwide and 9.6 million people succumbed to this disease. The most common cancer types affecting humans are: breast, colorectal, lung and prostate cancer (Bray et al., 2018). Apart from the enormous toll cancer has on the life of people it also has a major impact on the world economy where the total cost of cancer in Europe alone (in 2018) is estimated at €199 billion and approximately €70 billion as a result of productivity loss (Hofmarcher et al., 2020). All these crucial factors create a great incentive to devise novel cancer treatments and diagnostic methods.

How does cancer arise and what is our body's defence? One can imagine a cell as a well-tuned car that can only move within defined boundaries. Unfortunately, there are external ("meteor strike" ~ such as radiation) and internal ("engine breakdown" ~ such as errors done by the cell's replicative system) factors that can throw off the well-defined balance and push "the vehicle" away from its trajectory. This can lead to the activation of cellular oncogenes which can be considered "accelerators/gas pedals" that are always "on", forcing the cell to continuously divide. It also leads to the repression of its tumour suppressors, the first-line defence proteins of the cells, which function as "breaks"– stopping the cell from uncontrollably dividing thus halting its progression towards carcinogenesis. Knudson's pivotal research of retinoblastomas (intraocular cancer) (Knudson, 1971) led to the discovery of the first tumour suppressor gene, the retinoblastoma protein (pRb) that protects cells against neoplastic transformation (Friend et al., 1986; Fung et al., 1987). Since then the number of tumour suppressor has grown substantially, the most famous one being p53 protein.

There are several known "hallmarks" which define the nature of cancer cells. These consist of: self-sufficiency in growth signals, insensitivity to antigrowth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis and genome instability (Hanahan and Weinberg, 2000). This list was recently updated to also include active evasion from the immune system and metabolic reprogramming (Hanahan and Weinberg, 2011).

The observation that cancer cells' metabolism varies greatly from that of normal cells was described in pioneering work by Carl and Gerty Cori and Otto Warburg in the 1920s (Cori and Cori, 1925; Warburg et al., 1927). The eponymous "Warburg effect" describes a seemingly paradoxical situation where cancer cells in aerobic conditions prefer glycolysis, which is much less efficient in terms of ATP production, over oxidative phosphorylation. The role of this phenomena has since become a great centre of debate and several biological explanations have been offered to interpret the benefit of the Warburg effect in cancer cells. It has been suggested that the Warburg effect might be beneficial for cancer cells because it supports the excessive biosynthetic needs of proliferating cells. Even though glycolysis is a less efficient pathway for producing ATP and energy, it is more efficient in providing cells with biosynthetic building blocks, such as intermediates and substrates for the synthesis of

nucleotides, proteins and membrane components. Therefore, glycolysis is usually preferred by proliferative tissues and by cancer cells whereas oxidative phosphorylation is usually preferred by differentiated tissues. Another explanation investigates cellular energetics and ATP production; while it is true that oxidative phosphorylation is superior to aerobic glycolysis in terms of efficiency, the latter occurs at a much faster rate. Therefore, as evolutionary game theory models suggest, a higher rate of ATP production might have an advantage over higher yields because it provides quick access to a limited energy source. Different hypothesises look outside of the cell and take into consideration the adjacent environment – The Warburg effect might play a role in cell signalling via reactive oxygen species production (ROS) and support the tumour microenvironment via its acidification through increased lactate production (Liberti and Locasale, 2016). Even though all these hypothesises have sound logic behind them, the issue is yet to be conclusively resolved and more research is needed.

As the renewed interest in cancer metabolism has been gaining momentum in recent years, it has sparked interest in the role of metabolic enzymes in cancer. Mitochondria are the centre of many metabolic pathways, such as the tricarboxylic acid (TCA) cycle, urea cycle, fatty acid oxidation etc. They are also the main producer of energy, ROS (Chen et al., 2003) and play a key role in apoptosis (Zamzami et al., 1996), all of which are dysregulated in cancer. Therefore, some mitochondrial proteins might act as tumour promoters or tumour suppressors based on their effect on cancer metabolism. In this thesis I will provide an overview of the newly-found identities and mechanisms-of-action of eight of these mitochondrial tumour suppressors: proline oxidase, sirtuin 3 and 4, LACTB, succinate dehydrogenase, fumarate hydratase, mitochondrial tumour suppressor 1 (sometimes referred to as microtubule-associated scaffold protein 1) and tumour suppressor candidate 2 (also known as fusion protein 1). The role of these mitochondrial proteins in tumour suppression was recently discovered and our knowledge of their mechanism is still sparse and fragmentary.

2 Proline oxidase

Proline is a non-essential amino acid synthesised from glutamic acid. Thanks to its unique feature, i.e. having the nitrogen atom locked within a pyrrolidine ring, its Φ angle is constrained, which has a profound effect on protein conformation (MacArthur and Thornton, 1991). Proline is broken down by an enzyme called proline oxidase (POX), also known as proline dehydrogenase (PRODH), which is a mitochondrial inner-membrane bound enzyme (Brunner and Neupert, 1969). It is encoded by the PRODH1 gene, located at chromosome 22q11.21 (Campbell et al., 1997). POX catalyses the first reaction of proline catabolism: the oxidation of proline to Δ^1-pyroline-5-carboxylate (P5C). The two electrons produced by this reaction are transferred from FAD (a cofactor of POX) directly onto ubiquinone and subsequently to the third complex of the electron transport chain (ETC) (Hancock et al., 2016; Moxley et al., 2011; Wanduragala et al., 2010). P5C and its tautomeric form, glutamic-γ-

semialdehyde, which forms spontaneously, can be further converted into glutamate and ornithine (and vice-versa) thus bridging the TCA and urea cycle (Adams and Frank, 1980). Since the conversion of P5C back into proline requires NAD(P)H and proline can be re-oxidized in mitochondria by POX back to P5C, it has been suggested that this "proline cycle" can transfer reducing potential into mitochondria (Hagedorn and Phang, 1983; Fig. 1) hence maintaining redox homeostasis between mitochondria and cytosol (Phang et al., 1980). Recent works seem to support this hypothesis (Elia et al., 2017; Liu et al., 2015; Pandhare et al., 2009).
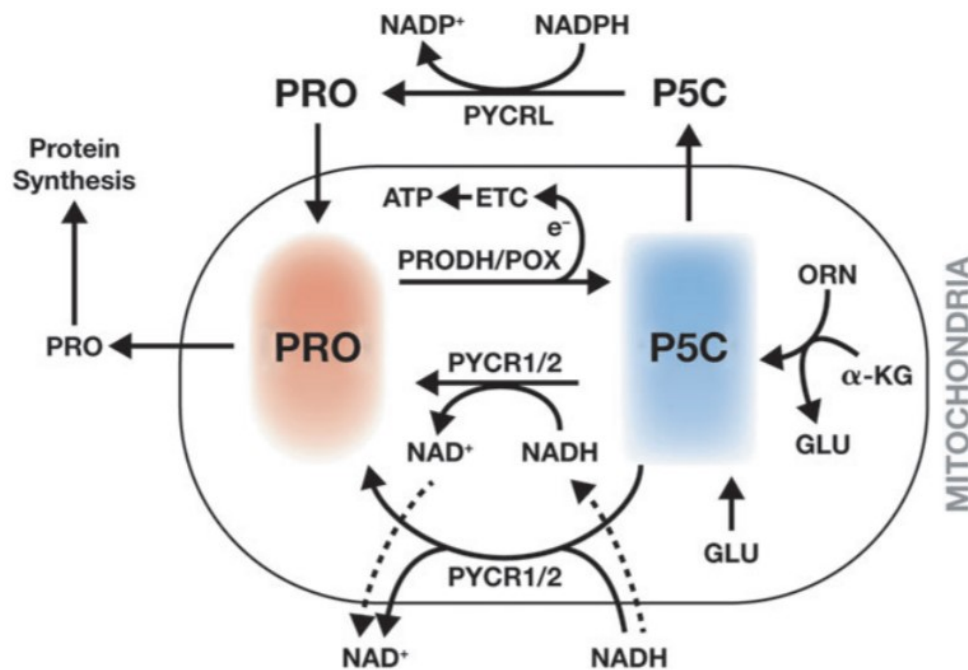

Figure 1: Hypothetical proline cycle. Cycling between proline and P5C can provide necessary NAD(P)+ for glycolysis and a pentose phosphate pathway (PPP), hence enhancing catabolism. Moreover, electrons produced by the oxidation of proline can be used to produce ATP. PYCR - pyrroline-5-carboxylate reductase. Adapted from Phang, 2019.

2.1 POX as a tumour suppressor

POX is also known as PIG6 (p53-induced gene 6) since its transcription is markedly enhanced by the expression of p53 (Polyak et al., 1997), a well know tumour suppressor and a transcription factor, that often acts through the induction of apoptosis in cancer cells (Finlay et al., 1989; Raycroft et al., 1990). This observation was later confirmed and expanded by showing that POX has p53 response elements both in promotor and intronic regions (Raimondi et al., 2013). To investigate whether POX can mediate apoptosis on its own, a p53-devoid cancer cell line (H1299) was transfected with green fluorescent protein (GFP) fused to POX. The GFP-POX transfected cells underwent significantly higher rates of apoptosis compared to cells only expressing GFP (Maxwell and Davis, 2000), suggesting a role of POX in inducing apoptosis in cancer cells. Based on multiple studies since the 2000s, POX's role as a tumour suppressor has been well recognized (Hu et al., 2007; Liu et al., 2006; Maxwell and Rivera, 2003). Immunohistochemical staining of 92 tumour/normal tissue pairs showed greatly reduced levels

of POX in the cancer cells from numerous tissues, especially the kidneys, colon, livers, rectum and pancreas (Liu et al., 2009).

POX's role as a tumour suppressor was further confirmed *in vivo* by using mouse xenografts injected with colon cancer cells with POX expression under Tet-off doxycycline-inducible (DOX) conditions. The (-) DOX group, which expressed POX protein, had fewer tumours compared to the (+) DOX group, where expression of POX was inhibited. The decrease of tumour size upon POX expression was shown to be mostly due to cell cycle arrest rather than apoptosis. This was confirmed by flow cytometry showing a decrease of S phase cells from 23% to 8-13% and an increase of G2 phase cells from 18% to 30-39% (Liu et al., 2009). Additional proof of ongoing cell cycle arrest was the inactivation of the cyclin-dependent kinase 2 (CDC2), that normally drives cell into mitosis (Nurse and Bissett, 1981). This was manifested by a POX-dependent increase in CDC2's Tyr15 phosphorylation and a decrease in Thr161 phosphorylation (Liu et al., 2009). Interestingly, POX has been shown to co-localize with another mitochondrial tumour suppressor (and a member of the ETC and TCA cycle) – Complex II, succinate dehydrogenase. POX and proline can down-regulate other ETC proteins (Complex I-IV) via a ROS-mediated pathway. In return, succinate acts as an uncompetitive POX inhibitor (Hancock et al., 2016).

2.1.1   POX and ROS production

How does POX mediate apoptosis? As we have previously discussed POX is involved in redox regulation and is directly connected with the ETC via ubiquinone. Mitochondria are the main cellular producers of ROS (Boveris and Chance, 1973; Chen et al., 2003; Kushnareva et al., 2002), which has a plethora of roles in cellular signalling and apoptosis (Ray et al., 2012). Therefore, some of the follow-up studies examined whether there is a possibility that POX exerts its tumour suppressive function through an increase in ROS production which would lead to the onset of apoptosis and growth arrest. This indeed was shown to be true in a study from 2001, which showed that the induction of POX in colon cancer cells increased ROS levels 2-fold leading to apoptosis (Donald et al., 2001). It was later reconfirmed as both, POX overexpression (Nagano et al., 2017) and GP120-induced POX expression (Pandhare et al., 2015), increased ROS generation. The POX-mediated apoptosis was abrogated when an antioxidant agent (superoxide dismutase) was introduced to colon cancer cells with induced expression of POX (Liu et al., 2005).

The role of ROS as a second messenger and its ability to mobilize Ca^{2+} is well established (Görlach et al., 2015). Calcineurin is a Ca^{2+}/calmodulin-dependent Ser/Thr protein phosphatase involved in many cellular signalling processes including apoptosis through BAD dephosphorylation (Wang et al., 1999) and interaction with Bcl-2, which disrupts NFAT (nuclear factor of activated T-cells) nuclear translocation (Shibasaki et al., 1997). As is the case with p53, POX have also been shown to activate calcineurin which led to the activation of apoptosis. POX antisense vector greatly diminished p53-

induced POX expression, the activation of calcineurin and subsequent apoptosis. Calcineurin inhibitor (FK-506) significantly reduced POX-dependent apoptosis, whereas p53-induced apoptosis decreased by approximately 30%, which seems to suggest that while the calcineurin pathway might be the primary pathway for POX-induced apoptosis, it is one of several possible mechanisms for p53-induced apoptosis (Rivera and Maxwell, 2005).

Apart from activating the intrinsic apoptotic pathways (via ROS), POX expression has been shown to stimulate expression of TNF-related apoptosis inducing ligand (TRAIL), DR5 and also cleavage of caspase-8, hinting that POX can also have a role in activating the extrinsic apoptotic pathway, possibly mediated via NFAT transcription factors (Liu et al., 2006). The peroxisome proliferation-activated receptor γ (PPARγ) agonist, troglitazone, can trigger TRAIL-mediated apoptosis in human lung carcinoma (Nazim et al., 2017) and also activate POX promotor in both PPARγ-dependent and independent fashion (possibly via p53) (Pandhare et al., 2006).

2.1.2 POX and inflammation

Prostaglandins are a biologically active group of compounds derived from arachidonic acid that have a multifaceted role in autocrine and paracrine signalling (Jabbour and Sales, 2004). One of their many tasks is to regulate inflammation, which is often associated with cancer progression, especially in colon cancer (Castellone et al., 2005; Holla et al., 2006).

Cyclooxygenase 2 (COX-2) can be found expressed in cancer and is responsible for prostaglandin synthesis (Maeng et al., 2014; Wu and Sun, 2015). Increased POX expression has been shown to notably reduce the levels of COX-2 as well as its main product – PGE$_2$ (prostaglandin E2). Restoring the levels of PGE$_2$ through external addition resulted in a drop of POX-mediated apoptotic levels from 19.1% to 7.7% and partially reversed the tumour growth inhibition. The reduction in COX-2 expression also seems to be ROS-mediated as manganese superoxide dismutase (MnSOD) antioxidant partially reversed POX's tumour suppressive effect (Liu et al., 2008). Furthermore, POX induction decreased the phosphorylation and activity of the epidermal growth factor receptor (EGFR) (a positive regulator of COX-2 activity (Lo et al., 2010)) and its downstream effectors while MnSOD partially reversed it, suggesting that POX expression might regulate COX-2 levels through its effects on EGFR signalling (Liu et al., 2008).

2.2 POX as a tumour survival factor

Hypoxia is a prominent feature of a tumour microenviroment which was shown to contribute to cancer progression. As the tumour mass rapidly expands, it inevitably outgrows the oxygen supply, which creates hypoxic regions within the tumour. Hypoxia then triggers a set of adaptive changes in the cell, which are orchestrated by the transcription factor called hypoxia inducible factor (HIF) (Wang and Semenza, 1993) (note: HIF can also be activated via oxygen independent mechanisms – by various oncogenes, tumour suppressors mutation or by inhibition of prolyl hydroxylase). These changes include

metabolic reprogramming – e.g. supressing the TCA cycle and regulating the transcription of glycolytic enzymes thus maintaining ATP production (Kim et al., 2006; Semenza et al., 1994), and lowering oxygen consumption by mitochondria, which rescues cells from hypoxia induced apoptosis (Papandreou et al., 2006). Furthermore, it has a role in neovascularization (Forsythe et al., 1996), epithelial-mesenchymal transition (EMT), invasion and metastasis (Krishnamachary et al., 2003; Yang et al., 2008).

It was shown that POX expression was increased under hypoxic conditions in multiple tumour cell lines *in vitro* as well as *in vivo* (mice xenograft model of human breast cancer). Moreover, hypoxia and low-glucose conditions seem to have an additive effect on its increased expression. *In vivo* experiments with human breast cancer xenografts expressing EGFP reporter gene under hypoxia-response elements promotor also showed a close correlation between the hypoxic regions and an increased POX expression. However, it seems that AMP-activated protein kinase (AMPK), rather than HIF-1α or HIF-2α, is responsible for the induction of POX expression (Liu et al., 2012) which is in agreement with the results of Pandhere et al. from 2009. This might suggest a link between POX and autophagy, since HIF-1 independent hypoxia autophagy signalling via AMPK was described by Papandreou et al. (2008). This theoretical link seems to be correct as both, POX knockdown and ROS scavengers, severely impaired the formation of autophagosomes and the conversion of LC3-I to LC3-II (Liu and Phang, 2012). Protective autophagy was shown to mediate cancer cell survival under stress conditions (Kinsey et al., 2019).

As we have already discussed, POX can have multiple roles in the cell: generating ROS, apoptosis and maintaining ATP levels in stress conditions. It seems that POX upregulation has a positive effect on cell viability during hypoxic/low-glucose conditions. Cells under low-glucose conditions alone decreased proliferation by 46.5% in contrast to hypoxia where the decrease was only 7.3%. The combination of these two resulted in a 73% decrease as well as a major drop in ATP production. Adding dehydroproline, a POX inhibitor, reinforced this effect, indicating POX's role in compensatory ATP production (Liu et al., 2012). When RKO, colon carcinoma, cells were treated with mTOR inhibitor, rapamycin, which simulates starvation, POX catalytic activity increased significantly as it did when glucose was withdrawn from the medium. More importantly, ATP levels were maintained or rebounded after a few hours of the initial stress induction, suggesting alternative substrates (such as proline) were used (Pandhare et al., 2009; Fig. 2). Interestingly, it seems that there is a difference in the metabolism of proline in 2D and 3D grown MCF10A H-RAS[V12] cells (derived from immortalized non-tumorigenic breast epithelial cells) and proline's role in metastasis. Cells growing in a 2D monolayer secreted proline, whereas cells growing in 3D spheroids used proline from the media, moreover, expression of POX increased by ~ 300% (during 3D spheroidal growth), while other enzymes of proline metabolism (pyrroline-5-carboxylate dehydrogenase, pyrroline-5-carboxylate synthase and pyrroline-5-carboxylate reductase 1) did not significantly change. Knockdown and inhibition of POX had a negative effect on

spheroidal growth (Elia et al., 2017). POX activity was also shown to be higher in metastases. When BALB/C mice with formed 4T1 breast cancer tumours were treated with L-tetrahydro-2-furoic acid (which was found to inhibit POX activity in 2D cultures) the quantity of lung metastases decreased in a dose-dependent manner (up to 60%) while the primary breast cancer tumour did not change (Elia et al., 2017).

To summarise, low levels of glucose with or without hypoxia lead to increased proline degradation, which can be used to maintain energy through increased ATP production. Similarly, hypoxia triggers protective autophagy (most likely via ROS signalization), which again can sustain a cell in stress conditions and under these specific conditions POX displays a tumour survival function.
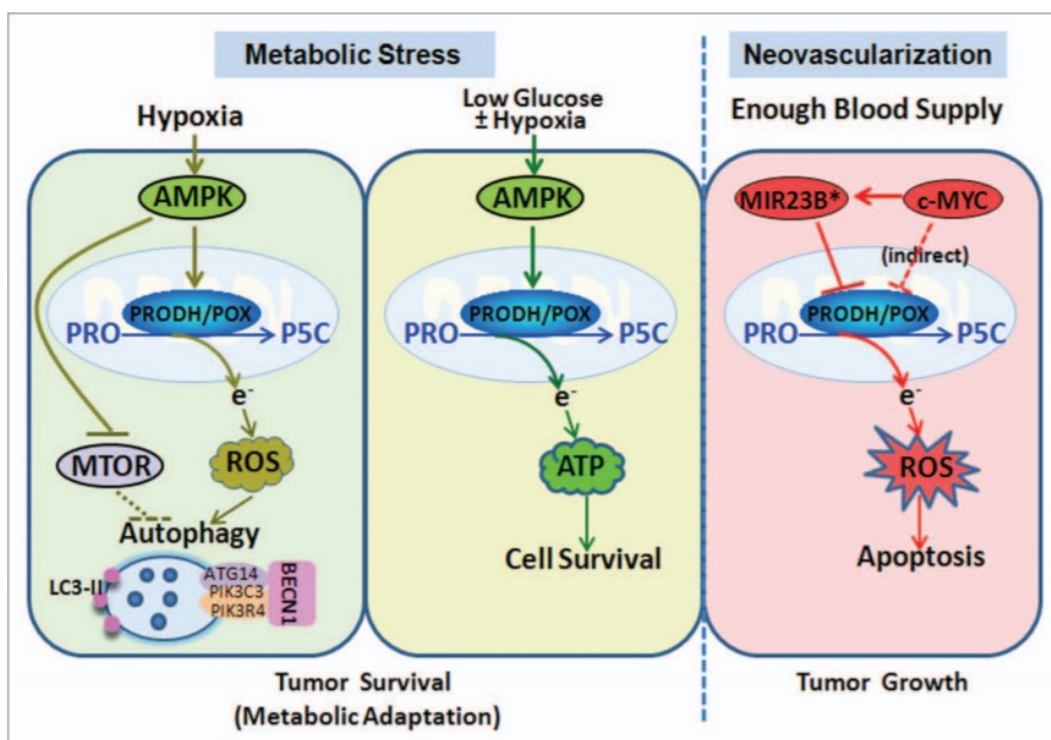

Figure 2: Under regular conditions POX acts as a tumour suppressor, initiating apoptosis via ROS generation. Under metabolic stress it is responsible for tumour survival. In low glucose conditions (regardless of hypoxia), POX participates in ATP production. During hypoxia, POX turns on protective autophagy. Adapted from Liu and Phang, 2012.

3 Sirtuins

Sirtuins are a family of highly conserved proteins that are found from prokaryotes to eukaryotes (Brachmann et al., 1995). Thus far, seven human sirtuins have been identified, three of which (SIRT3, SIRT4, SIRT5) were shown to be localized primarily in mitochondria (Du et al., 2018; Haigis et al., 2006; Iwahara et al., 2012; Scher et al., 2007; Schwer et al., 2002). Their main enzymatic activity is NAD$^+$-dependent deacetylase and mono-ADP-ribosyltransferase, although not all sirtuins share this activity, some reports have also shown desuccinylase, demalonylase, and other activities. Sirtuins are

involved in many cellular processes including senescence, immune response, apoptosis, regulating various metabolic pathways and have a greatly debated role in cancer (Dang, 2014).

3.1 Protein (de)acetylation

While both protein phosphorylation and acetylation were discovered within a short span of time (Fischer et al., 1959; Phillips, 1963), the latter was mostly ignored and has started to attract more attention in the past two decades. Soon after Phillips' discovery of histone acetylation, a correlation between transcription and histone acetylation has been recognized (Allfrey et al., 1964) and is now widely accepted. Since then, our knowledge of the acetylation's function has expanded greatly. It has been shown to affect the binding of transcription factors such as GATA-1 (Boyes et al., 1998) and p53 (Gu and Roeder, 1997) to DNA, and to alter the activity and function of many proteins such as heat shock protein 90 (HSP90) (Bali et al., 2005), α-tubulin (L'Hernaul and Rosenbaum, 1985) etc. The evolutionarily conserved regulatory role of lysine acetylation on individual proteins (that is on the ε-amino group) (Nakayasu et al., 2017) is becoming more and more recognized, as is the broad role of N-terminal protein acetylation (on the α-amino group of the first amino acid), which has been shown to be involved in protein stability, folding, interaction and localization (Aksnes et al., 2016).

A study of acetylated proteins within total liver proteome suggests that the majority of proteins, from the intermediate metabolism, is acetylated. It appears that lysine acetylation affects the activity of enzymes differently. For example: malate dehydrogenase seems to be activated by acetylation whereas argininosuccinate lyase is deactivated (Zhao et al., 2010). In prokaryotes such as *Salmonella Enterica* (de)acetylation controls carbon utilization and the usage of metabolic pathways. (De)acetylation requires NAD^+ and acetyl coenzyme A (Ac-CoA) respectively, both of which are involved in main metabolic pathways hence it can be used to sense the energy status of the cell (Wang et al., 2010).

3.2 SIRT3

SIRT3 gene has been mapped to the 11p15.5 region (Frye, 1999) and is expressed, to a various degree, in virtually all tissues (Fagerberg et al., 2014). While its mitochondrial localization has been confirmed in multiple studies (Lombard et al., 2007; Schwer et al., 2002), the nuclear localization remains fairly controversial (Cooper and Spelbrink, 2008; Iwahara et al., 2012; Sundaresan et al., 2008). Upon entry to mitochondria, SIRT3 is proteolytically processed to an active 28-kD product (Schwer et al., 2002). As the primary mitochondrial deacetylase, it has been shown to regulate the acetylation and thus activity of proteins such as: ATP synthase (Vassilopoulos et al., 2014), complex I (Ahn et al., 2008), Superoxide dismutase (Tao et al., 2010), Acetyl-CoA synthetase 2 (Schwer et al., 2006) and long-chain Ac-CoA dehydrogenase (Bharathi et al., 2013) thus exerting control over a multitude of processes such as aging, oxidative stress and energy metabolism (Brown et al., 2013; Someya et al., 2010).

3.2.1 SIRT3 as a tumour suppressor

When *SIRT3*^{−/−} mice are exposed to genotoxic stress, they display greatly increased ROS generation as well as chromosomal instability, compared to the WT SIRT3 mice. Both SIRT3 knockout (KO) mouse embryonic fibroblasts (MEFs) and the livers of *SIRT3*^{−/−} mice showed decreased mtDNA integrity. Even though, SIRT3 KO on its own is not sufficient for MEF immortalization, the cells exhibited lower levels of apoptosis when exposed to stress stimuli and most importantly, they could be immortalized by expression of a single oncogene (such as RAS or MYC), indicating SIRT3's role as a tumour suppressor. (Kim et al., 2010).

An analysis of 92 hepatocellular carcinoma (HCC) samples showed both, reduced levels of SIRT3 mRNA and protein, in contrast to non-tumour tissue (Zeng et al., 2017). 40-50% downregulation of SIRT3 in HCC was also observed in another study (Zhang and Zhou, 2012). Expression of SIRT3 in the HCC HepG2 cell line inhibited cell proliferation as well as reduced migration and invasion *in vitro*. It also led to reduction of Akt and PI3K protein levels whereas SIRT3 knockdown (in Huh7 HCC cells) led to upregulation, indicating its role in Akt/PI3K signalling (Zeng et al., 2017).

Gastric cancer (GC) also shows decreased levels of both SIRT3 mRNA and protein compared to adjacent normal tissue. SIRT3 overexpression slowed cellular proliferation and reduced colony formation numbers. These effects are possibly mediated through modulation of Notch-1 signalling as SIRT3 overexpression was accompanied by a reduction of Notch-1 mRNA and protein levels. siRNA-mediated knockdown of SIRT3, on the other hand, increased both levels of mRNA and expression of Notch-1. Overexpression of Notch-1 in GC cells partially reversed the previously discussed effects of SIRT3 expression (Wang et al., 2015). In addition, patients with low SIRT3 expression had a worse survival rate compared to those with high expression (Yang et al., 2014).

Similar tumour suppressive effects of SIRT3 were also shown in lung adenocarcinoma where SIRT3 was shown to be involved in p53 biology. Its overexpression upregulated p53 signalling, decreased oxidative stress, induced nuclear translocation of apoptosis inducing factor, induced apoptosis, and increased the ratio of Bax-Bcl-2 and Bad-Bcl-x/L (Xiao et al., 2013). Moreover, overexpression of SIRT3 was shown to downregulate levels of ubiquitin-ligase Mdm2 (involved in p53 degradation) thus stabilizing p53 protein (Zhang and Zhou, 2012).

Osteosarcoma H143B cell line and human colon carcinoma HCT116 had increased proliferation as well as enhanced HIF-1α (hypoxic) activation when SIRT3 was knocked down with shRNA (Bell et al., 2011). Additionally, SIRT3 was shown to be a mediator of Bcl-2-regulated and JNK2-regulated apoptosis in HCT116 cells (Allison and Milner, 2007). B-cell malignancies are also associated with the loss of SIRT3 and lower SIRT3 expression is connected with worse survival rates in cell lymphoma patients (Yu et al., 2016).

Various tumours are associated with iron metabolism dysregulation and iron deficiency (Ludwig et al., 2013). Iron serves as an essential cofactor in multiple proteins and is involved in processes such as cell cycle progression (Wang et al., 2004) and nucleotide synthesis (Barankiewicz and Cohen, 1987; Furukawa et al., 1992). SIRT3 was found to be involved in the regulation of cellular iron metabolism (Jeong et al., 2014). SIRT3 KO MEFs had increased transferrin receptor protein 1 (TfR1) expression (and subsequently iron uptake), which was mediated in a ROS-dependent (and HIF-1α-independent) manner. Specifically, it seems that ROS increased iron regulatory protein 1 (IRP1) binding activity, which then increased TfR1 expression. Iron regulatory protein 2 (IRP2) levels were also increased (Jeong et al., 2014). Indeed, both IRP1 (Pantopoulos and Hentze, 1998) and IRP2 (Hausmann et al., 2011) are confirmed to be regulated by oxidative stress. Transferrin receptor is strongly expressed in aggressive human pancreatic cancer (Ryschich et al., 2004), is upregulated by c-MYC oncogene, and was essential for cancer cell cycle progression and increased proliferation in human B lymphocyte cell line (P493-6) (O'Donnell et al., 2006). Interestingly, its expression is inversely correlated with SIRT3. Overexpression of SIRT3 in human pancreatic ductal adenocarcinoma cell line inhibited IRP1 activity and thus repressed TfR1 expression, which led to decreased proliferation (Jeong et al., 2014). SIRT3 KO MEFs exhibit faster proliferation (Finley et al., 2011) and knockdown of TfR1 abrogates this effect (as do Fe-chelators), further hinting at the importance of iron metabolism regulation in SIRT3's tumour suppressive activity (Jeong et al., 2014).

3.2.2 SIRT3 as an oncogene

In stark contrast to SIRT3's role in multiple cancer types above, comes a study of human melanoma cell lines (George et al., 2016). Both mRNA and protein levels of SIRT3 were much higher compared to normal human epidermal melanocytes and immortalized melanocytes. A similar increase was found when analysing human melanoma tissues. shRNA-mediated knockdown of SIRT3 in multiple melanoma cell lines resulted in decreased proliferation, inhibited colony formation and reduced migration capacity. Moreover, the knockdown cells exhibited senescence-like phenotype and dysregulation of the cell cycle – increase in G0/G1 phase cells. In a xenograft nude mouse model SIRT3 knockdown caused decreased tumour growth. When forced to express SIRT3, the Hs247T melanoma cell line exhibited increased proliferation and colony formation (George et al., 2016).

Another cancer type, where SIRT3 has a pro-proliferative role, is renal cell carcinoma (RCC). Levels of SIRT3 are significantly increased compared to normal cells and shRNA silencing of SIRT3 resulted in greatly reduced proliferation. Cells with catalytically inactive SIRT3 exhibited decreased tumorigenesis *in vivo*. SIRT3 was shown to have a role in glutamine-oxidation, as SIRT3 knockdown in RCC cells had impaired proliferation, glutamine oxidation and significantly reduced activity of glutamate dehydrogenase (without a change in GDH mRNA levels) (Choi et al., 2016). Both GDH and Isocitrate dehydrogenase 2, which is one of the key regulatory points of the TCA cycle, are known substrates of SIRT3 (Schlicker et al., 2008).

Mitochondrial targeting of p53 and its apoptogenic role in mitochondria has been well established (Marchenko et al., 2000; Mihara et al., 2003; Sansome et al., 2001; Talos et al., 2005). SIRT3 have been identified as rescuing cells from p53-induced senescence and growth arrest. SIRT3 interacts with p53's MASD (mitochondria-associated senescence domain) region between amino acid (AA) 64 and 209 and exerts deacetylation activity *in vitro* on p53 peptide sequences (Li et al., 2010).

3.3 SIRT4

SIRT4 is another mitochondrially localized member of the sirtuin family that has been shown to have a tumour suppressive effect (Ahuja et al., 2007; Sun et al., 2018). Even though SIRT4 is a member of the sirtuin family, which is mostly known for its deacetylation activity (Dang, 2014), it exhibits far stronger lipoyl- and biotinyl-lysine NAD^+-dependent removal activity (Mathias et al., 2014) and an ADP-ribosyltransferase activity (Ahuja et al., 2007).

The highest expression of SIRT4 is in the brain, heart, kidneys, liver and in the pancreatic β cells (Haigis et al., 2006). Through its ADP-ribosyltransferase activity SIRT4 interacts with glutamate dehydrogenase (GDH), which is inhibited by this modification (Haigis et al., 2006; Herrero-Yraola et al., 2001). Apart from GDH, SIRT4 associates with pyruvate dehydrogenase (PDH), oxoglutarate dehydrogenase and branched-chain alpha-keto acid dehydrogenase. SIRT4 attenuates the activity of PDH by hydrolysing the lipoamide cofactor from the dihydrolipoamide acetyltransferase component (of PDH). This component is responsible for transferring the acetyl group from pyruvate dehydrogenase's cofactor, thiamine pyrophosphate, to CoA (Mathias et al., 2014). In addition, mitochondrial SIRT4 interacts with pyruvate carboxylase and other acetylated biotin-dependent carboxylases (Wirth et al., 2013). In Pancreatic Islets of Langerhans (Ahuja et al., 2007) SIRT4 negatively regulates insulin secretion both *in* and *ex vivo* (Haigis et al., 2006). It interacts with the insulin-degrading enzyme and ATP/ADP translocase 2,3. Knockout of SIRT4 was shown to enhance insulin secretion (Ahuja et al., 2007) and to dysregulate fatty acid lipid metabolism (Laurent et al., 2013a, 2013b).

3.3.1 SIRT4 and cancer

SIRT4 is downregulated in gastric cancer and correlates with poor prognosis (Sun et al., 2018). Overexpression of SIRT4 inhibited proliferation of gastric cancer cells and downregulated expression of cyclin D and cyclin E thus inducing G1 cell cycle arrest (Hu et al., 2019). Apart from slowing proliferation, SIRT4 expression also inhibited migration and invasion and regulated matrix-metalloproteinase (MMP) 2 and 9. Furthermore, it upregulated E-cadherin expression and thus suppressed EMT, the pathway responsible for the migration and stem-ness properties of cancer cells. Protein levels of vimentin and N-cadherin, markers of mesenchymal state, were significantly lower with SIRT4 overexpression and higher with SIRT4 knockdown (Sun et al., 2018).

Another study pointing to the role of SIRT4 in tumour suppression came from the observations of interconnection between DNA damage and glutamine metabolism (Jeong et al., 2013). It was shown

that DNA damage leads to an unexpected decrease in glutamine uptake and in the intermediates of the TCA cycle and this decrease is necessary for efficient cell cycle arrest and DNA repair. As SIRT4 was shown to interact with the enzymes involved in the glutamine metabolism, the authors of this study examined the role of SIRT4 in DNA damage. Interestingly, SIRT4 mRNA levels were highly induced (15-fold) upon different types of DNA damage. This also occurred in p53-inactive and p53-null cells, suggesting it was a p53-independent event. Indeed, it was shown that SIRT4 represses the metabolism of glutamine into TCA cycle allowing the cell to undergo growth arrest and DNA repair (Jeong et al., 2013; Fig. 3). SIRT4 knock-out cells showed more aneuploidy and polyploidy and were able to form colonies in the absence of glucose. Furthermore, SIRT4 knock-out mice were more likely to develop tumours than wild type (WT) mice and the formed tumours had greater weight and volume (Jeong et al., 2013). The cAMP-response element binding protein 2 (CREB2), which is under the control of mTORC1, has been found to affect the transcription SIRT4, which in turn controls the activity of GDH. The decreased GDH activity affected the energy metabolism, lowered the ATP/ADP ratio and, after glucose deprivation, showed a notable increase in cell death. SIRT4 also reduced tumour development and mean tumour volume mass in a *Tsc2*^{-/-} mouse embryonic fibroblasts (MEFs) xenograft model. Loss of SIRT4 is associated with a shorter time of metastasis in breast, colon, bladder, ovarian, gastric and thyroid carcinomas (Csibi et al., 2013).
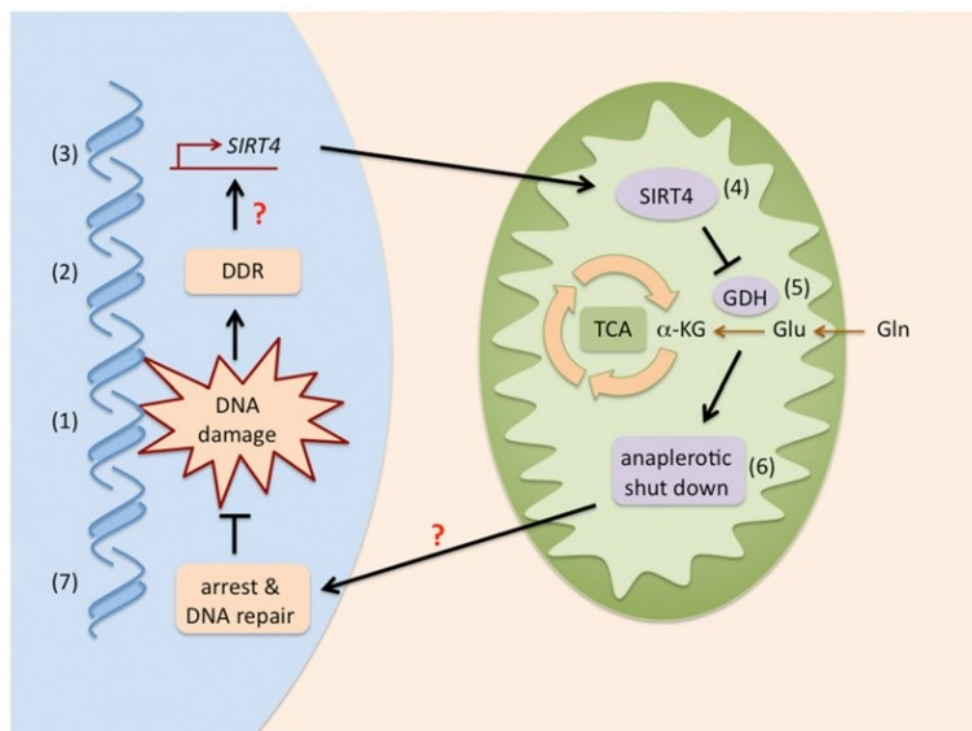

Figure 3: DNA damage response leads to higher expression of SIRT4. Increased SIRT4 activity inhibits GDH, thus blocking anaplerotic replenishment of the TCA cycle, which results in DNA repair. Adapted from Fernandez-Marcos and Serrano, 2013.

4 LACTB

Serine beta-lactamase-like (LACTB) protein is one of the latest additions to the growing number of mitochondrial tumour suppressors, its role in cancer was first described in 2017 (Keckesova et al., 2017).

LACTB is derived from bacterial penicillin-binding proteins/β-lactamases (Peitsaro et al., 2008; Smith et al., 2001) and was introduced to eukaryotic cells through endosymbiosis. Its orthologs are found in all vertebrates (with completed genome) as well as in *Caenorhabditis elegans, Ciona intestinalis, Schistosoma japonicum* etc., sharing active sites and signature motifs (Peitsaro et al., 2008). In humans it is localized in the 15q22.1 chromosome and is expressed in all human tissues tested thus far with the strongest expression in skeletal muscle and the liver (Smith et al., 2001).

In bacteria, β-lactamases are involved in the synthesis of peptidoglycan, the major component of a bacterial cell wall. However, metazoans do not possess cell wall structures, leaving the role of LACTB in metazoans unknown. In *C. elegans* LACT-1 (a homologue of LACTB) is upregulated in response to fungal infection, therefore it was proposed that LACTB might be used as a sensor of fungal infection (Pujol et al., 2008). Human LACTB was found to localize into the intermembrane space of mitochondria with the N-terminal 97 AA being essential for mitochondrial targeting. It was shown to polymerize into filaments in the intracristal regions, suggesting a structural role within mitochondria (Polianskyte et al., 2009). In a 2008 study, LACTB was identified, together with Lpl and Ppm1l, as one of the three novel genes associated with obesity. Transgenic mice with upregulated LACTB levels varied notably from the control mice, their fat-mass-to-lean-mass ratio being 20% higher than wild type control (Chen et al., 2008). This would suggest LACTB plays a role in obesity and fatty acid metabolism (Chen et al., 2008; Yang et al., 2009). LACTB was also shown to be associated with mitochondrial ribosome (Koc et al., 2001).

4.1 LACTB as a tumour suppressor

LACTB was recently shown, through *in vitro*, *in vivo* and clinical studies, to have a tumour-suppressive function in breast cancers (Keckesova et al., 2017). Its protein levels were decreased in many breast cancer cell lines and analysis of clinical human breast cancer samples showed 34-42% decrease of LACTB expression compared to normal tissues. LACTB overexpression in several breast cancer cell lines had a negative effect on their proliferation while non-tumorigenic cells were not negatively affected. Most importantly, induced expression of LACTB in already formed tumours had a negative effect on the growth of these tumours – tumour growth was reduced significantly after 2-3 weeks of LACTB expression with many tumours disappearing completely. In contrast to the control tumours, the tumours where LACTB was induced displayed more differentiated epithelial-like morphology. This was confirmed by *in vitro* studies where epithelial cancer cells with LACTB induction formed epithelial islands and had increased levels of epithelial differentiation markers (EPCAM, CD24)

and decreased CD44, ZEB1 and other mesenchymal markers. Their proliferation rate and tumorigenesis were also decreased. Knocking down levels of LACTB in normal human epithelial cells in combination with over-expression of oncogene (such as HRAS^{G12V}, or MYC^{T58A}) led to malignant transformation of these cells with resulting tumour formation further confirming the tumour-suppressive role of LACTB. Additional mechanistic studies showed that LACTB induces differentiation and loss of tumorigenicity of breast cancer cells through the reprogramming of mitochondrial lipid metabolism. This is achieved through the downregulation of the lipid-synthesizing mitochondrial phosphatidylserine decarboxylase (PISD) enzyme, which leads to changes in the levels of mitochondrial lipids: lysophosphatidyl-ethanolamine and phosphatidylethanolamine (Keckesova et al., 2017; Fig. 4).
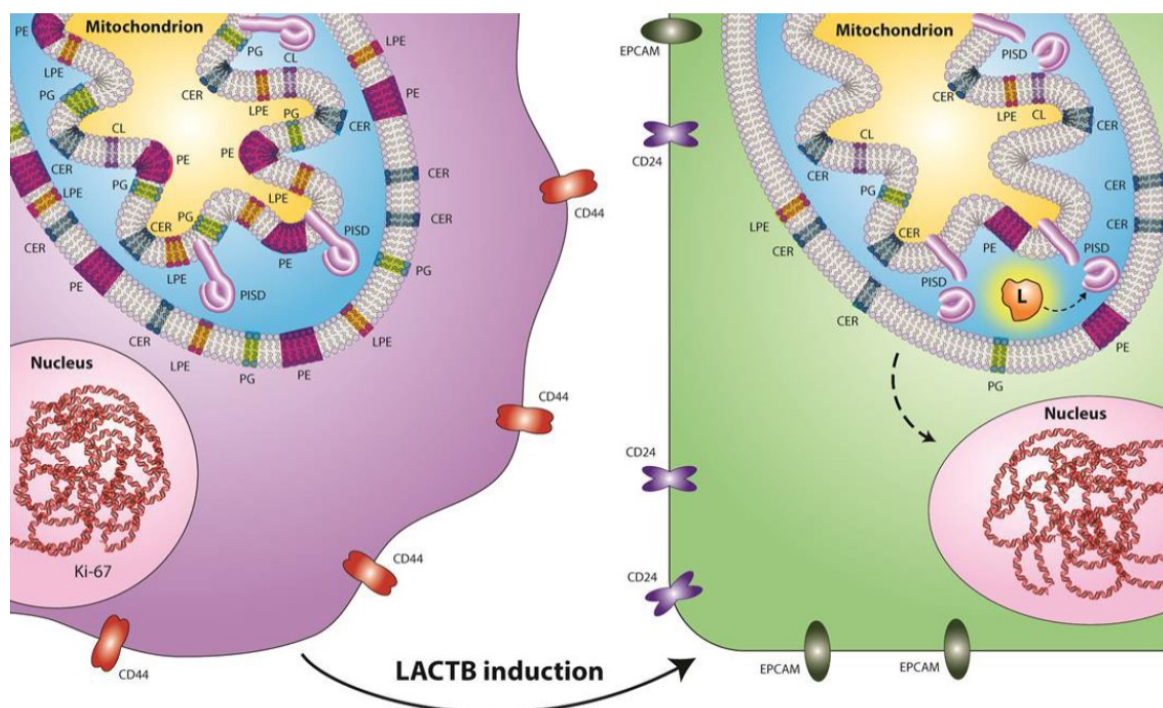

Figure 4: Induced expression of LACTB leads to multiple changes in cancer cells i.e. a decrease in expression of proliferation markers and an increase in the expression of differentiation markers. This is most probably achieved by LACTB's ability to decrease the expression of PISD and consequent changes in the composition of mitochondrial lipids. Adapted from: Keckesova et al., 2017

The role of LACTB in differentiation was also shown in normal cells, namely in skeletal myogenesis. In C2C12 myoblast that were induced to differentiate, LACTB levels gradually increased. LACTB was shown to be negatively regulated by miR-351-5p, whose expression was decreased upon LACTB induction. Overexpression of miR-351-5p led to downregulation of LACTB. When LACTB was silenced, the proliferation rate of C2C12 myoblasts increased leading to offset of differentiation, while silencing LACTB during differentiation prevented its completion (Du et al., 2019).

Similar results, supporting the tumour-suppressive role of LACTB, were also found in another study performed in colorectal cancer cells (CRC) (Zeng et al., 2018). Both, LACTB expression and

mRNA levels, were markedly reduced in CRC which was attributed to promotor methylation and hypoacetylation of H3 histone. These findings suggest that LACTB downregulation in CRC is regulated epigenetically. LACTB overexpression slowed down the proliferation rate, delayed G1/S transition, induced apoptosis and reduced the migration and invasiveness of CRC cells. Moreover, LACTB also inhibited tumour growth in mouse xenograft. A similar morphological change, pointing to differentiation, was also observed in CRC cells, where epithelial-cell markers were increased and mesenchymal-cell markers decreased upon LACTB expression. LACTB co-localized with tumour suppressor p53 and p53 levels were post-transcriptionally increased with LACTB overexpression. The interaction of LACTB with p53 was further supported by the fact that in cell lines with mutated TP53 (HT29 - CRC, SW480 – colon adenocarcinoma), LACTB could not suppress tumorigenicity. LACTB's deletion mutants and co-immunoprecipitation studies showed that LACTB interacts with p53's C-terminal domain. LACTB overexpression prolonged the half-life of p53 from 15 minutes up to 60 minutes (Zeng et al., 2018). p53 is mostly degraded via Mdmd2-mediated ubiquitination and C-terminal domain of p53 is essential for this effect (Carter et al., 2007; Lang et al., 2014). Therefore, the authors of this study examined whether LACTB might modulate the stability of p53 protein. Indeed, LACTB overexpression inhibited the ubiquitination of p53 and prevented Mdm2 from binding to p53, thus rescuing p53 from degradation. Moreover, both p53 inhibitor, pifithrin-α, and p53 siRNA attenuated the tumour suppressive effects caused by LACTB overexpression, confirming that in CRC LACTB acts through its effect on p53 protein (Zeng et al., 2018).

The role of LACTB in tumour suppression was also recently reported in HCC by Xue et al. where overexpression of LACTB led to a decrease of tumour growth *in vivo* and to an inhibition of proliferation, migration and invasion *in vitro* (Xue et al., 2018). In glioma cells, overexpression of LACTB inhibited proliferation, invasion and angiogenesis as well as expression of MMP2,9, proliferating cell nuclear antigen and vascular endothelial growth factor (Li et al., 2019).

4.2   LACTB as a prognostic factor

LACTB mRNA is downregulated in HCC patients. Genes negatively correlated with survival were more abundant with low expression whereas genes positively related with survival were more expressed with high LACTB levels, hence LACTB expression can be considered as a biomarker of poor prognosis (for HCC) (Xue et al., 2018). A similar situation applies for CRC, where LACTB expression can be considered an independent prognostic factor for poor cancer survival (Zeng et al., 2018). Significantly decreased LACTB expression was also found in gliomas (Li et al., 2019).

5 Other mitochondrial tumour suppressors

Several additional tumour suppressors were described as functioning inside mitochondria even though these reports are very limited in scope with largely unclear mechanism. Nevertheless, they do offer us a broader picture of the variety of mitochondrial proteins with a (possible) tumour suppressive function.

5.1 Succinate dehydrogenase

SDH, also known as succinate:ubiquinone oxidoreductase or mitochondrial respiratory Complex II, is a TCA cycle enzyme as well as a member of the ETC. It is made up of four subunits – Flavoprotein (Fp also known as subunit A - SDHA), iron-sulphur protein (Ip or subunit B - SDHB) and two transmembrane - anchor – proteins (Succinate-ubiquinone oxidoreductase cytochrome B large subunit – cybL for short or SDHC and Succinate-ubiquinone oxidoreductase cytochrome b small subunit, cybS, or SDHD) (Sun et al., 2005).

5.1.1 SDH as a tumour suppressor

Twenty eight out of 35 cases of hemangioblastoma (benign vascular tumours of the central nervous system) exhibited a negative or weak diffuse pattern of SDHB expression (Roh et al., 2019) while mutations of SDHC caused autosomal dominant paraganglioma (type 3) (Niemann and Muller, 2000). Lower expression of SDHA in breast cancer was associated with diagnosis at an earlier age. Both SDHA and SDHB -negative cancers correlated with a lower histological grade. In general, about 3.19% of breast cancer patients exhibited mutation in SDHA and 0.1% in SDHB (Kim et al., 2013). Mutations in SDHD were also identified as causing hereditary paraganglioma (Baysal et al., 2000). SDHB subunit mutations cause susceptibility to familial paraganglioma and (familial) pheochromocytoma (Astuti et al., 2001). Two SDHD mutations, Asp92Tyr and Leu139Pro, were found to cause almost all cases of hereditary paragangliomas in the Netherlands, moreover, the maternal wild type SDHD allele was lost in those patients (Taschner et al., 2001). Mitochondria with mutated SDHD had altered morphology – loss of cristae, presence of inclusion bodies and a swollen appearance (Douwes Dekker et al., 2003).

While heterozygous germline mutations in SDHB, SDHC, SDHD have been identified as causing hereditary paragangliomas and pheochromocytomas, up until 2010 no mutation in SDHA subunit was identified that would be connected with paraganglioma/pheochromocytoma. In 2010, SDHA mutation, Arg589Trp, was detected and confirmed to be a loss-of-function mutation (Burnichon et al., 2010).

5.1.1.1 Mechanism

How are defects of the TCA cycle enzymes connected to tumorigenesis? To begin with, SDH-inhibited cells accumulated succinate which in turn can inhibit the activity of HIF prolyl-hydroxylase.

This can lead to the stabilization of HIF-1α and the start of its transcription program (Selak et al., 2005; Fig. 5).

In ovarian cancer there is a decrease in SDH activity and SDHB has altered expression. SDHB knockdown resulted in faster proliferation and greater colony-forming ability in agar in C1 mouse ovarian cancer cells. Human ovarian cancer cells (HEY) where SDHB was knockdown exhibited morphological change – they displayed an elongated spindle-like shape. mRNA analysis showed upregulation of several transcription factors involved in EMT. SDHB knockdown also seems to orchestrate a transcriptional program that promotes (or maintains) histone methylation, most probably via the S-Adenosyl methionine cycle. The hypermethylation then leads to the induction of EMT. As SDH is intertwined with carbon metabolism, it might not come as a surprise that SDHB knockdown also severely altered the levels of TCA cycle intermediates as well as AA, nucleotides and intermediates of PPP and glycolysis – Glucose was diverted towards PPP an nucleotide biosynthesis, while the TCA cycle was sustained by glutamine metabolism, which became crucial for the cell's survival (Aspuria et al., 2014).
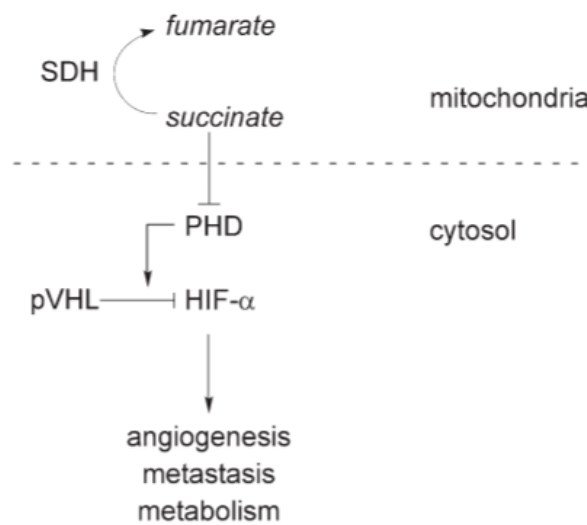

Figure 5: Summary of succinate's role in signalling. Dysfunctional SDH leads to an accumulation of succinate, which inhibits PHD that subsequently leads to HIF stabilization and turning on the pseudohypoxic transcription program. Adapted from: Selak et al., 2005

Another study examined the characteristics of a transgenic mouse cell line with mutated SDHC (SDHC E69). These cells displayed higher ROS production, DNA damage and apoptosis. Cells that escaped from apoptosis underwent transformation and were able to form small benign tumours. Taken together, it seems that mutations in SDHC can lead to cellular transformation through its effect on ROS production and the resulting genomic instability (Ishii et al., 2005). Furthermore, 3.8-fold induction of SDHC is sufficient to induce apoptosis and SDHC-deficient cells are resistant to proapoptotic drugs and Fas receptor (Albayrak et al., 2003)

5.2 Fumarate hydratase

Another member of the TCA cycle, fumarate hydratase (FH), catalyses reversible hydration of fumarate to malate. Apart from being an intermediate of the TCA cycle, fumarate is involved in cellular signalling; its accumulation leads to dysregulation of iron metabolism and increases ferritin gene transcription, which promotes proliferation (Kerins et al., 2017). It also acts as a competitive inhibitor of HIF prolyl hydroxylase, which leads to HIF stabilization (Isaacs et al., 2005) and has been suggested to act as a so-called oncometabolite, a small molecule, the accumulation of which leads to metabolic dysregulation and allows future cancer progression (Yang et al., 2012).

5.2.1 Fumarate hydratase in cancer

Heterozygous germline missense mutation in FH predisposes to multiple cutaneous and uterine leiomyomatosis syndrome (Alam et al., 2005). FH mutations are also present in inherited uterine fibroid, skin leiomyomata and papillary renal cancer (Tomlinson et al., 2002). Interestingly, in gastric cancer, the inhibition of FH led to a better response to cisplatin-mediated chemotherapy. The higher FH expression in GC tissues negatively correlated with the prognosis and could be considered as a reliable indicator for cisplatin treatment (Yu et al., 2019). While patients with germline FH mutation have been diagnosed with other malignancies such as breast cancer, it seems FH is not a breast cancer predisposing gene (Kiuru et al., 2005).

FH-defective fibroblasts and renal cells are protected from apoptotic death. This occurs through the activation of AMPK upon the loss of FH activity. This might happen through the fumarate accumulation and modulation of the activity of proteins from the Bcl-2 family (which regulate apoptosis) (Bardella et al., 2012). FH knockdown also resulted in increased expression of MET oncogene via stabilized HIF-1α, the expression of MET can then, in turn, stabilize HIF-1α thus creating a forward feeding loop. Consistently with other reports, FH knockdown in MEF cells also makes them resistant to apoptosis (Costa et al., 2010).

Another study, performed in kidney and renal cancer tumours and cell lines, showed the role of the iron-sensing pathway in FH-mechanism. Inactivation of FH in these cells, contrary to the previous study, decreased the levels of AMPK and p53 tumour suppressor. Reduced AMPK levels lowered the expression of the divalent metal transporter 1 iron transporter, which led, through the activation of the iron regulatory proteins, IRP1 and IRP2, to increases in expression of the hypoxia inducible factor HIF-1α. This ultimately contributed to increased oncogenic growth of FH-deficient cells (Tong et al., 2011). The interconnection of FH-deficiency and HIF1-α stabilization was further confirmed by another study examining renal cancers. This study showed that inactivating mutations of FH result in the glucose-mediated generation of cellular ROS and ROS-dependent HIF-1α stabilization (Sudarshan et al., 2009).

5.3 MTUS1/MTSG1

MTUS1, microtubule-associated scaffold protein 1, also known as mitochondrial tumour suppressor 1, is a novel mitochondrial tumour suppressor gene that was discovered in 2003 and is located at chromosome 8p21.3-22 (Seibold et al., 2003). It encodes five isoforms also known as Angiotensin-II type 2 receptor-interacting proteins (ATIPs) (Di Benedetto et al., 2006). While it is ubiquitously expressed in normal tissue, mRNA expression was not detectable in pancreatic tumour tissues and cell lines (Seibold et al., 2003). MTUS1's major transcripts are ATIP1, ATIP3, ATIP4 with different tissue distribution – ATIP1 and ATIP4 in the brain, the latter more in the cerebellum and fetal brain, ATIP3 in the prostate, bladder, ovary, colon, and breast (Di Benedetto et al., 2006). While other isoforms exhibited tumour suppressive effects (Rodrigues-Ferreira et al., 2009) and were reduced in multiple cancer types (Zhao et al., 2015; Zuern et al., 2010), only ATIP1, also known as isoform 5, has been proven to be associated with mitochondria, specifically, with the outer mitochondrial membrane. In endothelial cells, MTUS1 was shown to play a role in regulating mitochondrial motility and morphology through its interaction with mitofusins (Wang et al., 2018). It was also shown to exert an anti-inflammatory role and to regulate cytokine production, mainly through p38 mitogen-activated protein kinases and NF-κB (Wang et al., 2016).

The expression of MTUS1 in oral tongue squamous cell carcinoma is significantly reduced, especially in poorly differentiated cases (with ATIP1, ATIP3a and ATIP3b isoform being reduced the most) which correlated with poor survival. ATIP1 re-expression led to slower proliferation, G1 arrest and apoptosis and was accompanied by upregulation of p53 and reduced phosphorylation of extracellular signal-regulated kinase (ERK1/2) (Ding et al., 2012). Tumour suppressor p53 has been shown to interact with ATIP1 promotor and p53 knockdown led to decreased ATIP1 expression (Chen et al., 2011). Expression of ATIP1 inhibited insulin and basic fibroblast growth factor signalling cascades as well as epidermal growth factor-induced ERK2 activation and cell proliferation. The presence of angiotensin II receptor type 2 was required for these effects (Nouet et al., 2004).

5.4 FUS1/TUSC2

A set of 19 genes with a possible tumour suppressor function have been identified in a 630 kb lung cancer homozygous deletion region on chromosome 3p21.3. These genes showed rather an infrequent mutation rate, suggesting they might represent new examples of haploinsufficient genes, where one functional copy of the gene is not sufficient for it to function properly and to prevent carcinogenesis (Lerman and Minna, 2000). Among other genes from this region, FUS1/TUSC2 (tumour suppressor candidate 2 also known as fusion protein 1) expression was able to alter the cell cycle, induce apoptosis, reduce tumour growth both *in vitro* and *in vivo*, as well as inhibit metastasis (Ji et al., 2002). Interestingly, FUS1 knockout cells had activated c-Abl protein (Lin et al., 2007), a non-receptor tyrosine kinase, whose role encompasses processes such as proliferation, adhesion, migration, survival and

apoptosis (Sirvent et al., 2008). While the mechanism of FUS1 action remains unknown, it has been shown that it can reduce and inhibit Abl tyrosine kinase (Lin et al., 2007).

FUS1 is downregulated also in soft-tissue and bone sarcomas (Li et al., 2011), lung cancer (Prudkin et al., 2008) as well as in pleural malignant mesothelioma (Ivanov et al., 2009). miR-663b, which is notably upregulated in nasopharyngeal carcinoma (the levels increasing with advancing stages), was found to target FUS1 thus negatively affecting its expression (Liang et al., 2017). FUS1 expression was found to be inhibited by several secondary structural elements on the 3' untranslated region and by two small open reading frames in the 5' untranslated region (Lin et al., 2011).

Additional evidence to the possible tumour suppressor role of FUS1 is that non-small lung cancer cell lines (NCI-H1299, NCI-H322) overexpressing FUS1 exhibited about a 75% decrease in colony formation and an increased doubling time. While no signs of apoptosis were observed, G1 arrest was detected in H1299 (Kondo et al., 2001). Expression of FUS1 also inhibited tumour growth in lung cancer, reduced metastasis and prolonged survival *in vivo* (Ito et al., 2004). Co-expression of FUS1 and p53 has a synergistic effect, moreover, Mdm2 expression was downregulated, which led to increased accumulation of p53 (Deng et al., 2007). Interestingly, myristoylation of FUS1 was found to be essential for its tumour suppressive effects, stability and subcellular, mitochondrial localization (Uno et al., 2004).

It seems that FUS1 plays a role in oxidative stress and nutrient/energy sensing as well as inflammation. FUS1 KO mice showed pathologies in antioxidant, mTOR and PTEN/AKT pathways. Furthermore, they showed signs of an early hearing decline, which was restored by antioxidant treatment. Cochlear tissue staining of aging FUS1 KO mice showed signs of chronic inflammation (Tan et al., 2017). Curiously, chronic inflammation was also detected in $FUS1^{-/-}$ mice after exposure to asbestos, which could, incidentally, downregulate FUS1 RNA levels in intraperitoneal immune infiltrates of WT mice. $FUS1^{-/-}$ mice also experienced activation of inflammatory, genotoxic and antioxidant stress response proteins when exposed to asbestos. Asbestos-induced alteration in cytokine production was FUS1-dependent. Taken together, this study showed that FUS1 provides a link between mitochondrial homeostasis and inflammation (Uzhachenko et al., 2012). Furthermore, FUS1 upregulated IL-15 and altered expression of more than 40 genes engaged with the immune system (Ivanova et al., 2009). FUS1 KO mice developed signs of autoimmune disease, had increased formation of spontaneous vascular tumours and finally, had defects in NK cell maturation that were connected to low IL-15 expression (Ivanova et al., 2007).

6 Conclusion

Mitochondrial tumour suppressors are a relatively new area of research with limited and fragmentary sources of information. This study describes mitochondrial tumour suppressors the function of which has been confirmed by three or more independent studies. As the research progresses, we will inevitably find and characterize more of them allowing us to learn about novel vulnerabilities of cancer cells that can ultimately be used to design new cancer treatments.

To sum up, even though mitochondrial tumour suppressors are a diverse group of proteins, it seems that they share some underlying characteristics. Firstly, several of them either directly (POX, SDH, FH) or indirectly (SIRT3, SIRT4) participate in metabolic pathways and in iron metabolism (SIRT3, FH), showing the importance of metabolic dysregulation in cancer. Even though the remaining two proteins discussed in this thesis (FUS1, MTUS1) have not been that well explored, it seems that they are involved in modulating the immune response, which is currently a widely researched topic in the cancer. Whether it is the double-edged role of cytokines in carcinogenesis, the role of inflammation or the possible ways of immunotherapy such as: interferon treatment, cancer vaccines, antibodies administration (immune checkpoint blockade), adoptive cellular therapy, oncolytic viruses and others, this area is enjoying more and more attention (Farkona et al., 2016; Smyth et al., 2004). Secondly, most of them are connected to ROS generation as well as HIF signalling. The cellular reprogramming triggered by (pseudo)hypoxia and neovascularization are important elements of tumour development. Thirdly, several of them are connected to the notorious p53 tumour suppressor – either being induced by it (POX, MTUS1) or directly interacting with it (LACTB, SIRT3). In addition, some of them have a synergistic effect or correlate with the higher p53 level (FUS1, FH). As p53 is mutated in a large number of cancers, these interactions could prove to be crucial in bettering our concept of tumour suppression in general. Finally, and interestingly, the role of these tumour suppressor proteins is not always clear cut as they can, based on the context, also promote tumour growth and act as survival factors showing that the context of these results is a very important variable that should be always taken into consideration.

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Secondary sources are marked with an asterisk (*).

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