A new view on the functions of the lysine demalonylase activity of SIRT5

Jarmila Nahálková,*[a] – Biochemistry, Molecular, and Cell Biology Unit; Biochemworld co., Snickar-Anders väg 17, 74394, Skyttorp, Uppsala County, Sweden; Mobile: +46 73 7707700; E-mail: jarmila.nahalkova@biochemworld.net; ORCID ID: https://orcid.org/0000-0001-9918-6105

* - corresponding author

Abstract

The substrate specificity of the pharmaceutically attractive tumor-promoter SIRT5 was already investigated multiple times by advanced proteomic tools. However, the present pathway enrichment and gene function prediction analysis bring new highlights to the overall knowledge about the lysine demalonylation activity of SIRT5, a member of the sirtuin family with many roles in aging and age-related diseases. It shows previously unreported aspects of the involvement of the lysine demalonylated SIRT5 substrates in Eukaryotic translation elongation (ETE), Amino acid and derivative metabolism (AADM), and Selenoamino acid metabolism (SAM). The cluster of the elongation factors (EEF1A1, EEF2, EEF1D, and EEF1G) belonging to ETE participates in the peptide chain elongation and the export of the tRNA-s from the nucleus the primary sites of the proteosynthesis. SIRT5 regulates key enzymes with tumor-promoting functions involved in AADM (GLUD1, SHMT1, ACAT1), which fits its experimentally proven tumor promoter functions. In contrast, SIRT5 also lysine demalonylates tumor suppressor substrates involved in AADM and SAM (ALDH9A1, BHMT, GNMT). It indicates comparable functions like directly interacting family member SIRT3, which was reported to have dual tumor promoter/oncogene functions. Similar to the roles of the sirtuins, the SAM pathway impacts longevity, protects against cardiovascular diseases, and is associated with hepatic steatosis. The selen supplementation also mediates the calorie restriction effect, which increases the NAD+/NADH ratio in the cells and stimulates the expression of SIRT5 and other sirtuins. SIRT5 in turn regulates the selenocysteine synthesis through the lysine demalonylation of the participating ribosomal proteins, SECISBP2 and GNMT, which creates a regulatory loop.

Keywords: SIRT5; SIRT3; tumor suppressor; tumor promoter; lysine malonylation; pathway enrichment; eukaryotic translation elongation; selenocysteine

Abbreviations: calorie restriction – CR; KO – knockout; Nicotinamide Adenine Dinucleotide – NAD+; Nicotinamide Adenine Dinucleotide + Hydrogen – NADH; pyruvate dehydrogenase – PDH; succinate dehydrogenase – SDH; Tricarboxylic acid cycle – TCA cycle; WT – wild type;

Introduction

SIRT5 is a member of the NAD+-dependent sirtuin family with multiple deacylation activities. The unique character of SIRT5 within the family is its participation in reprogramming the cellular metabolism for the metabolic demands of fast-proliferating tumor cells. Unsurprisingly, this feature attracted the scientific community's attention to identify the main factors contributing to the function.

SIRT5 expression is essential for cancer cell growth, such as in acute myeloid leukemia (AML) cells since the downregulation inhibits AML development in mouse models (Yan et al., 2021). Its upregulation during the tumor cell transformation supports cancer cell proliferation (Wang et al., 2018) (Greene et al., 2019). The genetic deletion of SIRT5 however, does not have any significant impact on the metabolism of the experimental mice except for only 60 % postnatal survival out of the expected Mendelian proportion (Yu et al., 2013). It suggests, that SIRT5 functions in the knockout mice (KO) are not essential in surviving animals, but lost SIRT5 can cause 40 % lethality in the embryonal stage.

SIRT5 possesses several enzymatic activities including weak deacetylase (Nakagawa et al., 2009; Ogura et al., 2010), but stronger desuccinylase (Jintang et al., 2011) (Park et al., 2013), deglutarylase (Tan et al., 2014), and demalonylase activities (Peng et al., 2011). Instead, the most significant lysine deacetylation function in mitochondria is fulfilled by SIRT3 (Lombard et al., 2007). About 80 % overlap exists between the lysine acetylation and succinylation processed by SIRT5 in mitochondria (Rardin et al., 2013).

According to the former studies, the succinylome processed by SIRT5 is linked to the typical mitochondrial functions, such as the tricarboxylic acid (TCA) cycle, oxidative phosphorylation, amino acid degradation, fatty acid oxidation, ketone bodies production, and glycolysis/gluconeogenesis (Du et al., 2018; Park et al., 2013; Tan et al., 2014). Activities of succinate dehydrogenase (SDH; respiratory complex II) and pyruvate dehydrogenase (PDH) are increased in SIRT5 KO mice, and an increased rate of respiration is observed in the presence of SDH and PDH substrates. It suggests, that SIRT5 inhibits the activity of the respiratory chain complex driven through these enzymes. Previously, the desuccinylation substrate interaction network showed functions in the respiratory chain complexes I, III, and IV, glutathione-S-transferase, ribosomes, and chaperonin-containing T complex 1 (TCP1) enzymes (Park et al., 2013). The pathway enrichment further exhibited involvement in the functions of frataxin, and the nuclear HMGB1-HMGB2-HSC70-ERP60-GAPDH complex, which can recognize the non-natural nucleoside (thiopurine) incorporation in DNA (Krynetski et al., 2003). Enriched was also SDH-mABC1-PIC-ANT-ATPase complex containing structural and functional subunits of the mitochondrial ATP-sensitive K+ channel (Park et al., 2013), which further demonstrated the functional involvement of SIRT5 lysine desuccinylation activity.

An interesting question can be raised about the functional differences between the specificity of the lysine succinylome, malonylome, and glutarylome regulated by SIRT5. Previously identified pathways linked to the lysine demalonylation activity of SIRT5 do not differentiate from the desuccinylation activity since SIRT5 lysine malonylome is also associated with fatty acid degradation, glycolysis/gluconeogenesis, TCA cycle, and oxidative phosphorylation (Du et al., 2018). The gluconeogenesis, glycolysis, and urea cycle as the main target of SIRT5 lysine demalonylation activity was also determined by the study by Nishida et al. (2015). The study claims that the expression of SIRT5 can increase the energy flux through glycolysis by the processing of appropriate substrates (Nishida et al., 2015). Similarly, the bioinformatics analysis of the lysine deglutarylation substrates of SIRT5 also emphasized functions in the metabolism of cellular respiration, amino acids, and fatty acids metabolism (Tan et al., 2014).

On the subcellular level, the SIRT5 succinylome is mostly localized in the mitochondria, cytoplasm, and nucleus (Park et al., 2013), while the malonylome can be typically found in the cytoplasm (Nishida et al., 2015) (Du et al., 2018) and mitochondria (Nishida et al., 2015). The cytoplasmic demalonylation should assist in increasing glycolysis/gluconeogenesis, while the mitochondrial desuccinylation serves to the intensification of oxidative phosphorylation with the increased fatty acid metabolism and diminished glycolysis/gluconeogenesis pathway (Nishida et al., 2015) (Du et al., 2018). SIRT5 is important for cancer cell metabolism since the metabolisms of glucose and fatty acids are vital for cancer growth.

The present study was capable to retrieve new information about less studied lysine malonylome regulated by SIRT5 employing the pathway enrichment and gene function prediction analysis. The interaction network of the SIRT5 substrates is helpful in the building of a new working hypothesis about the main molecular mechanisms of the SIRT5 lysine demalonylation activity in

cells with special attention to cancer cell metabolism. It complements the current knowledge with the support of the published data and gives new ideas for further experimental research.

Materials and methods:

The list of substrates lysine demalonylated by SIRT5: Substrates were collected from the study by Nishida et al. (2015). For the selection of the substrates was used increased threshold with False Discovery Rate (FDR) values lower or equal to 0,01 and KO/WT (knockout/wild-type) ratio > 1,5. It yielded a substrate list of 69 proteins (Tab. 1S; Gene column 7-76), which was subjected to the pathway enrichment analysis.

Pathway enrichment and gene function prediction analysis: Names of SIRT5 substrates not recognized directly were converted to GeneMania compatible symbols using Protein Knowledgebase Uniprot (Bairoch et al., 2005; Consortium, 2021) and Online Catalogue of Human Genes and Genetic Disorders (OMIM ®) (Hamosh et al., 2000). GeneMania (3.5.2) analysis (Franz et al., 2018; Warde-Farley et al., 2010; Zuberi et al., 2013) was performed under Cytoscape (3.9.1) environment (Cline et al., 2007) (Lopes et al., 2010) utilizing the substrate list including SIRT5 as a query (Table 1S, column Gene 7-76). The analysis was run against the *H. sapiens* database (update 29-04-2021) and by including all types of interaction networks. The analysis was set up for the identification of the top 20 related genes and at the most 20 attributes by employing the standard weighting method of GeneMania. It represents query gene-based weighting, which is identical to Gene Ontology (GO) Biological Processes. GO Cellular Component-based weighting also produced the main results described by the study, however with different scores. GO Molecular Function-based weighting yielded fewer results than other

weighting methods (Metabolism and Metabolic pathways; Translation, and Cellular responses to stress). Scores (weights) express the predictive value, that GeneMania assigns to the pathways, and it is a measure of how well they correspond to the query dataset compared to the non-query.

Artwork: The illustration was drawn using Adobe Illustrator Artwork 26.0 and Inkscape 1.1.1.

Results and discussion

The tumor promoter activity of SIRT5 linked to the adaptation of the cell metabolism for the optimal growth of cancer cells is an attractive subject for tumor biologists. Determination of the targets of SIRT5 enzymatic activity leading to increased cancer cell growth might offer an alternative approach for pharmaceutical interventions.

The present study provides new information about the molecular mechanisms mediating the tumor promoter activity of SIRT5 by the pathway enrichment analysis of the lysine malonylome processed by SIRT5. For this purpose, it was used the list of the lysine malonylated substrates of SIRT5 mined from the study (Nishida et al., 2015), which applied the label-free quantitative proteomics in the combination with the affinity enrichment of liver lysates from WT and SIRT5 KO mice. The pathway enrichment analysis using Ingenuity Pathway Analysis (IPA) suggested that the substrates demalonylated by SIRT5 are functionally involved in the regulation of the energetic flux through glycolysis (Nishida et al., 2015). Glycolysis/gluconeogenesis are the main targets of the demalonylation activity of SIRT5 according to other authors (Du et al., 2018).

The present analysis uses a hypothesis, that the proteins interacting directly *via* protein-protein interactions are with a high probability of participating in the same cellular and molecular functions. Mutations in the directly interacting disease genes lead to similar disease phenotypes

(Oti, 2006). It also uses a central model of the interaction network medicine, which relies on targeting multiple essential proteins since the treatment development for the disease pathologies through just a single node appears not feasible (Nolan, 2007). The most promising drug-targeting strategies are based on the partial inhibition of several protein nodes within the interaction network instead of the complete inhibition of a single node (Csermely et al., 2005). The targeting of several nodes has practical implications for the development of therapeutical approaches and the present analysis attempts to define them.

The performed analysis based on the GeneMania *H. sapiens* database (updated 29-4-2021) identified the most significant pathways associated with the interaction network of substrates lysine demalonylated by SIRT5 previously not detected pathways. Excluding the general metabolic pathway, they include Eukaryotic translation elongation (ETE), Amino acid and derivative metabolism (AADM), and Selenoamino acid metabolism (SAM), which share proteins nodes belonging to ribosomal proteins (Fig. 1, Table 1). The clustering and visualization of the substrate nodes assisted in the identification of tumor promoter/suppressor functions of the nodes associated with these pathways. I also revealed new information about the subcellular localization of SIRT5 lysine demalonylation activity.

Proteosynthesis

In addition to ribosomal proteins, the remaining substrates connected to the ETE cluster are subunits of the eukaryotic elongation factor complex (Fig. 1; EEF1A1, EEF2, EEF1D, EEF1G). They are participating in the peptide chain elongation when aminoacyl-tRNA binds to site A, delivers single amino acid to the growing polypeptide chain, and together with tRNA-s dissociates from the ribosome. A mutation in the aa-tRNA binding domain of EEF1A1 (EF1A)

causes the accumulation of the tRNA in the nucleus and it proposes its function in the export of the tRNA-s from the nucleus to the primary sites of the proteosynthesis, ribosomes at the rough endoplasmic reticulum (ER) (Grosshans et al., 2000).

Amino acid and derivative metabolism (AADM)

SIRT5 acts as a tumor promoter (Greene et al., 2019) (Wang et al., 2018), and the functions of its lysine malonylated substrates involved in the amino-acid metabolism support these conclusions.

It is known, that besides glucose metabolism, the metabolism of glycine and glutamine are the most important promoters of cancer cell growth (Jain et al., 2012) (Wang et al., 2018). Glutamine metabolism regulated by SIRT5 is increasingly utilized in proliferating tumor cells. Desuccinylation and stabilization of glutaminase (GLS), an enzyme catalyzing the conversion of glutamine to glutamate strongly promotes tumor cell growth (Greene et al., 2019).

SIRT5 also lysine demalonylates several crucial enzymes involved in cancer cell metabolism, which confirms its regulatory role. AADM cluster includes several enzymatic nodes with functions confirming the tumor-promoting effect of SIRT5 (Fig. 1, Tab. 1). SIRT5 promotes the growth of colorectal cancer cells by deglutarylation and activation of L-glutamate dehydrogenase 1 (GLUD1; Fig. 1, Cluster AADM), an enzyme catalyzing the enzymatic conversion of glutamate into α-keto glutarate, an anaplerotic substrate of the TCA cycle. In this way, GLUD1 increases TCA flux and supports the anabolic metabolism of the cancer cells (Wang et al., 2018). GLUD1 is also deacetylated and activated by SIRT3 (Schlicker et al., 2008), another member of the sirtuin family with the function in cancer, which shares multiple substrates with SIRT5. The lysine demalonylation of GLUD1 by SIRT5, however, remains to be examined.

The accelerated tumor growth is also supported by the activity of Serine hydroxymethyltransferase (SHMT1; Fig. 1, Cluster AADM), an enzyme catalyzing the conversion of serine and tetrahydrofolate to glycine, and 5, 10-methylene tetrahydrofolate (Schirch, 1982) required for thymidine and purine synthesis (Zeng et al., 2021). The enzyme exists as cytoplasmic (SHMT1) and mitochondrial (SHMT2) isoforms (Garrow et al., 1993) playing fundamental roles in cancer cell proliferation with certain crosstalk between the actions of the isozymes (Paone et al., 2014).

A cytoplasmic isoform SHMT1 is a tumor promoter with a central role in the metabolic reprogramming of the cancer cells and adaptation to the tumor microenvironment (Jain et al., 2012), which fits SIRT5 functions (Greene et al., 2019). The glycine uptake and its mitochondrial metabolism are particularly increased in the actively proliferating cancer cells. Targeting glycin dependency as a major supply of carbon e.g. by the downregulation of SHMT1 is a strategy for cancer treatment (Jain et al., 2012). The knockdown initiates the cell cycle arrest at G1-phase and p53-induced apoptosis, which occurs due to uracil misincorporation during DNA replication. Compared to SHMT2 downregulation, the cell cycle arrest effect is much stronger, which confirms that SHMT1 is more important for lung cancer cell survival than SHMT2 (Paone et al., 2014). Mitochondrial isozyme SHMT2 promotes the growth of the cancer cells by limiting pyruvate kinase and redirecting the glucose metabolism towards glycolysis (Kim et al., 2015). Interestingly, SMHT2 is also acetylated, succinylated, and fatty-acylated. These modifications are removed by SIRT3 (Wei et al., 2018), SIRT5 (Yang et al., 2018), and HDAC11 (Cao et al., 2019), which indicates additional functional regulations.

Another tumor promoter present in the AADM cluster is mitochondrial Acetyl-CoA acetyltransferase (ACAT1; Fig. 1), an enzyme with a tumor growth-supporting function in

prostate cancer (Guan et al., 2020). SIRT5 acts as a prostate cancer promoter due to the upregulation of the MAPK pathway through ACAT1 (Guan et al., 2020). SIRT5 positively regulates the expression of ACAT1 in PC3 cells and reversely, ACAT1 positively regulates the expression of SIRT5 in LNCaP cells. Additional evidence that ACAT1 is a demalonylation substrate of SIRT5 (Nishida et al., 2015) provides a study, which detected the physical interaction of ACAT1 with SIRT5 (Guan et al., 2020).

Contrary to the mentioned results, additional nodes of the AADM cluster shows tumor suppressor functions. Aldehyde dehydrogenase 9 family member A1 (ALDH9A1; Fig. 1) oxidizes a broad range of aldehyde substrates to the corresponding acids such as γ-aminobutyraldehyde to γ-aminobutyric acid (Kaisaki et al., 2014). Its deficiency causes DNA damage leading to genomic instability and potential tumorigenesis (Jung et al., 2022).

Another tumor suppressor node is Betaine-homocysteine S-methyltransferase (BHMT, Fig. 1, AADM), which processing by SIRT5 is also not characterized yet. The cytosolic enzyme catalyzing the production of methionine and dimethylglycine from betaine and homocysteine (Garrow, 1996) is involved in the degradation of choline to L-serine, one-carbon methylation, trans-sulphuration, cysteine, and methionine metabolism (Teng et al., 2011). BHMT KO mice are susceptible to fatty liver and hepatocellular carcinoma, which indicates tumor suppressor functions and a protective role against hepatic steatosis. In addition, ALDH9A1 and BHMT are also deacetylation substrates of SIRT3 (Kaisaki et al., 2014), which further confirms the connectivity of the SIRT3 and SIRT5 protein-protein interaction network of tumor suppressors/promoters.

A central enzyme involved in the ammonia detoxification through the urea cycle Carbamoyl phosphate synthetase 1 (CPS1; Fig. 1; AADM), is activated after SIRT5 deacetylation

(Nakagawa et al., 2009) (Ogura et al., 2010) and deglutarylation (Tan et al., 2014). Since CPS1 is also a demalonylation substrate (Nishida et al., 2015), it indicates that demalonylation by SIRT5 also regulates CPS1 activity. The evidence that SIRT5 regulates the urea cycle through the first involved enzyme CPS1, was provided based on the hyperammonemia in the blood occurring in SIRT5 KO mice accompanied by deficiency of CPS1 activity (Nakagawa et al., 2009). It could, however, occur through the mentioned three post-translational modifications and not only through its deacetylation as it was originally assumed.

The present analysis revealed an AADM subcluster containing enzymes with tumor growth-promoting (GLUD1, SHMT1, ACAT1) and tumor suppressor activities (ALDH9A1 and BHMT). The occurrence of shared substrates with the tumor suppressor/oncogene SIRT3 proposes, that the common network of SIRT3 and SIRT5 substrates modulate the cellular microenvironment through both tumor suppressor/promoter functions.

Selenoamino acid metabolism (SAM)

A new pathway linked to SIRT5 lysine demalonylase activity identified by the present study is the Selenoamino acid metabolism (Fig. 1, Tab. 1). Despite the functional relationships between the selen levels and the sirtuin family function were not studied in-depth (Alehagen et al., 2021), there exist strong indications about mutual effects.

Selenocysteine can mimic the effect of calorie restriction (CR) and extend the lifespan in nematodes (Kim et al., 2018), which fits the functions of the sirtuin family. In addition, selenocysteine decreases the high-glucose and reactive oxygen species (ROS) toxicity, which also imitates the effect of CR. Selenium supplementation regulates DNA methylation and has a protective effect on DNA, chromosome damage, telomere length, and mitochondrial DNA

(Ferguson et al., 2012). Further, it decreases the effect of one of the hallmarks of Alzheimer´s disease, Aβ, and the associated toxicity (Kim et al., 2018), which occurs by the inhibition of NF-κB transcription through the deacetylation of RelA/p65 (Yeung et al., 2004).

Besides the functional link to CR, another common feature of the selenoprotein metabolism and SIRT5 is a connection to hepatic steatosis syndrome. Mice lacking selenocysteine lyase (ScLy), an enzyme contributing to the recycling of selenium for selenoprotein synthesis, develop a whole range of metabolic syndrome symptoms including hepatic steatosis (Seale et al., 2012). SIRT5 also deacetylates metabolism-related proteins, which decreases hepatic steatosis in ob/ob mice, an obesity mouse model (Du et al., 2018). In addition, overexpression of SIRT5 in the obesity ob/ob mice model attenuates the hepatic steatosis symptoms (Du et al., 2018) (Fig. 2). It can be concluded that the mechanism of the selenium effect on hepatic steatosis is by increasing the NAD+/NADH ratio, which further stimulates SIRT5 activity (Fang et al., 2015) (Fig. 2). The results indicate that the selenoprotein metabolism and SIRT5 could be linked with metabolic pathways leading to the hepatic steatosis by the lysine demalonylation of the appropriate substrates.

Except for the ribosomal proteins, SAM and AADM share only two nodes, Selenocysteine insertion sequence (SECIS)-binding protein (SBP2) and glycyl N-methyltransferase (GNMT) (Fig. 1). SBP2 binds to the selenoprotein mRNA and mediates the insertion of selenocysteine into the polypeptide chain (Copeland et al., 2000), and GNMT catalyzes the synthesis of N-methyl glycine from glycine by transferring methyl residue from S-adenosylmethionine. It is a tumor suppressor, which maintains genomic integrity by participating in the biosynthesis of purines and pyrimidines, preventing uracil misincorporation, and increasing DNA repair (Wang

et al., 2014). Colon and liver GNMT is downregulated by feeding selen-deprived feed to rat models (Uthus et al., 2006), which displays the functional link to the selen metabolism.

Interestingly, methylselenocystein (MSC) can suppress the negative effect of the cancerogenic N-methyl-N-nitrosourea on the circadian cycle and it occurs by restoring the NAD^+/NADH ratio and SIRT1 activity (Fang et al., 2015) (Fig. 2). MSC addition increases the expression of SIRT1, which deacetylates AcBMAL1, decreases its binding to the E-box element of the promoter of PER2 and rises its circadian transcription (Fang et al., 2015), (Hu et al., 2011), (Fig. 2). It further stimulates circadian expression of the genes involved in the DNA repair and suppression of cancerogenesis (Fang et al., 2015). The regulation of the circadian cycle also occurs through the SIRT3-FOXO3-CLOCK pathway (Fig. 2) (Nahálková, 2022); (Tomita, 2017); (Chaves et al., 2014); (Tong et al., 2016); (Jacobs et al., 2008) where FOXO3 is a crucial regulator of CLOCK and SIRT3 is a direct SIRT5 interactor (Pourhaghighi et al., 2020) (Ahuja et al., 2007).

Deacetylation and dessucinylation of substrates involved in mitochondrial bioenergetics are related to heart functions. There also occurs a potential link between the blood selenium levels, expression of SIRT5, and cardiac disease (Fig. 2). Interestingly, the low blood plasma levels of selenium are associated with low expression of sirtuins SIRT1, SIRT5, SIRT6, and SIRT7 and upregulation of C-reactive protein (CRP), a biomarker of the increased risk of cardiovascular disease (Salonen et al., 1982) (Suadicani et al., 1992) (Giacconi et al., 2021). SIRT5 KO mice also suffer increased size of heart infarction and the effect is linked to the SIRT5 processed succinylome, which is widely spread in heart tissues (Boylston et al., 2015) (Sadhukhan et al., 2016). SIRT5 re-expression in SIRT5 KO mice protects against death due to cardiac pressure overload (Hershberger et al., 2017). The maintenance of NAD+ levels in the heart can prevent cardiac failure (Pillai et al., 2010). It occurs through the activation of the LKB1 by SIRT3

deacetylation, which further stimulates the LKB1-AMPK pathway (Pillai et al., 2010). SIRT3 protects the mitochondrial homeostasis against cardiomyocyte apoptosis and the development of diabetic cardiomyopathy by activating Parkin-mediated mitophagy (Yu et al., 2017).

Selenium levels, SIRT3 and SIRT5 display a common functional link to hepatic steatosis and cardiovascular disease. Selenocysteine mimics CR and affects the expression of several sirtuins including SIRT5, which represents a strong functional connection. The present analysis completed the functional loop by linking the functions of the lysine malonylated substrates of SIRT5 to the selenoamino acid pathway, which mimics CR, impacts longevity, protects against hepatic steatosis and cardiovascular disease, and affects several sirtuins expression.

Subcellular localization of the lysine demalonylation substrates

The majority of the common protein nodes involved in three interaction subnetworks ETE, AADM, and SAM are ribosomal proteins (Fig. 1), which do not correspond with the generally accepted exclusively mitochondrial subcellular localization of SIRT5.

The extramitochondrial localization is supported by the previously reported existence of two isoforms. The SIRT5iso1 occurs both in the cytoplasm and mitochondria of vertebral cells, while SIRT5iso2 is localized in the mitochondria of primates (excluding humans) (Matsushita et al., 2011). Another study of SIRT5 succinylome reported a portion of SIRT5 substrates localized in the cytoplasm of cell lines and mouse liver tissue and a minor proportion in the nucleus. In addition, the study also detected ribosomal proteins processed by SIRT5 desuccinylation activity (Park et al., 2013). Processing of the histone substrates with potential impact on the chromatin functions proposes the nuclear localization of SIRT5 (Park et al., 2013). The presence of histones H2BC4 and H1-0 on the substrate list (Tab. 1S) as a part of the

interaction network Cellular Response to Stress and External Stimuli (Tab. 1) also supports the existence of the nuclear lysine demalonylation activity of SIRT5. Low H1-0 expression is essential for tumor cell growth since its silencing allows the subset of the cancer cells to proliferate indefinitely. On the other hand, its re-expression suppresses the oncogenic pathways and limits cancer cell proliferation (Torres et al., 2016), which is therapeutically interesting. The potential regulatory effect of the lysine demalonylation of H1-0 could represent another mechanism of the tumor promoter/suppressor activity of SIRT5, which remains to be elucidated. In conclusion, the present bioinformatic analysis suggests that substrate demalonylation occurs not only in mitochondria and cytoplasm but also in ribosomes and the nucleus.

Interaction with SIRT3

SIRT5 and SIRT3 process multiple substrates which suggest they share related cellular and molecular functions. The influence of SIRT3 and SIRT5 on the growth of the cancer cells and the protective effect against cardiac disease and hepatic steatosis are likely mediated by their direct interaction and shared substrates.

SIRT5 with high confidence interacts with SIRT3 (Pourhaghighi et al., 2020) (Ahuja et al., 2007), a tumor suppressor, which genetic deletion triggers the whole range of diseases including cancer, neurodegenerative and cardiovascular diseases, and metabolic syndrome (McDonnell et al., 2015) (Nahálková, 2022). SIRT3 functions as a tumor suppressor in gastric cancer cell lines by downregulation of Notch (Wang et al., 2015) (Haigis et al., 2012). Recently, the dual tumor suppressor/oncogenic functions were shown to be mediated by SIRT3 (Chen et al., 2014). The suppressor/promoter effect of SIRT3 and SIRT5 on the growth of cancer cells raises curiosity to discover the differences in the substrate specificity of these two family members.

Conclusion

The present pathway enrichment and gene function prediction analysis lead to several conclusions with a potential impact on future experimental research. It creates ideas and a strong working hypothesis for further scientific investigations, which would not be possible to obtain through conventional literature and data-mining methods.

It shows, that the interaction network of the lysine malonylated substrates of SIRT5 is related not only to the primary glucose metabolism and mitochondrial bioenergy production. The demalonylation of the elongation factors further suggests the functions in the peptide chain elongation and the transport of tRNA-s from the nucleus to the ribosomal location in the cytoplasm.

It also affects amino acid metabolism, which involves several enzymes with tumor promoter and suppressor activities. The substrates lysine demalonylated by SIRT5 increase cancer cell growth by regulation of the key enzymes involved in AADM (GLUD1, SHMT1, ACAT1), which explains the tumor promoter function of SIRT5. Interestingly, SIRT5 also processes the tumor suppressor substrates involved in AADM and SAM (ALDH9A1, BHMT, GNMT). The available data indicate a likely connection to both tumor promoter and suppressor functions, which might depend on the cellular microenvironment conditions. The final effect of the demalonylation on cancer cell growth, however, remains to be investigated.

Selenoamino acid metabolism, which also affects CR and the functions of other members of the sirtuin family is a novel pathway linked to SIRT5 lysine demalonylation activity. Involvement in the selenocysteine metabolism is a new feature of SIRT5, despite the effect of

methylselenocystein on the increasing NAD+/NADH ratio mimicking calorie restriction effect is already known. The ratio stimulates the activity of several members of the SIRT family including SIRT5, which through the lysine demalonylation of the appropriate enzymes further controls the selenocysteine metabolism and creates a regulatory loop.

Acknowledgments

The research was conducted from the financial resources of Biochemworld co., Uppsala County, Sweden.

Conflict of interest

There is no conflict to declare.

Consent statement/Ethical approval

Not required.

References

Ahuja, N., Schwer, B., Carobbio, S., Waltregny, D., North, B.J., Castronovo, V., Maechler, P., Verdin, E., 2007. Regulation of insulin secretion by SIRT4, a mitochondrial ADP-ribosyltransferase. J. Biol. Chem. 282, 33583–33592. https://doi.org/10.1074/jbc.M705488200

Alehagen, U., Opstad, T.B., Alexander, J., Larsson, A., Aaseth, J., 2021. Impact of selenium on biomarkers and clinical aspects related to ageing. A review. Biomolecules 11, 1–14. https://doi.org/10.3390/biom11101478

Bairoch, A., Apweiler, R., Wu, C.H., Barker, W.C., Boeckmann, B., Ferro, S., Gasteiger, E., Huang, H., Lopez, R., Magrane, M., Martin, M.J., Natale, D.A., O'Donovan, C., Redaschi, N., Yeh, L.-S.L., 2005. The Universal Protein Resource (UniProt). Nucleic Acids Res. 33, D154–D159. https://doi.org/10.1093/nar/gki070

Barbarese, E., Koppel, D.E., Deutscher, M.P., Smith, C.L., Ainger, K., Morgan, F., Carson, J.H., 1995. Protein translation components are colocalized in granules in oligodendrocytes. J. Cell Sci. 108, 2781–2790. https://doi.org/10.1242/jcs.108.8.2781

Boylston, J.A., Sun, J., Chen, Y., Gucek, M., Sack, M.N., Murphy, E., 2015. Characterization of the cardiac succinylome and its role in ischemia-reperfusion injury. J. Mol. Cell. Cardiol. 88, 73–81. https://doi.org/10.1016/j.yjmcc.2015.09.005

Cao, J., Sun, L., Aramsangtienchai, P., Spiegelman, N.A., Zhang, X., Huang, W., Seto, E., Lin, H., 2019. HDAC11 regulates type I interferon signaling through defatty-acylation of SHMT2. Proc. Natl. Acad. Sci. U. S. A. 116, 5487–5492. https://doi.org/10.1073/pnas.1815365116

Chaves, I., van der Horst, G.T.J., Schellevis, R., Nijman, R.M., Koerkamp, M.G., Holstege, F.C.P., Smidt, M.P., Hoekman, M.F.M., 2014. Insulin-FOXO3 signaling modulates circadian rhythms via regulation of clock transcription. Curr. Biol. 24, 1248–1255. https://doi.org/10.1016/j.cub.2014.04.018

Chen, Y., Fu, L.L., Wen, X., Wang, X.Y., Liu, J., Cheng, Y., Huang, J., 2014. Sirtuin-3 (SIRT3), a therapeutic target with oncogenic and tumor-suppressive function in cancer. Cell Death Dis. 5, 1–7. https://doi.org/10.1038/cddis.2014.14

Cline, M.S., Smoot, M., Cerami, E., Kuchinsky, A., Landys, N., Workman, C., Christmas, R., Avila-Campilo, I., Creech, M., Gross, B., Hanspers, K., Isserlin, R., Kelley, R., Killcoyne,

S., Lotia, S., Maere, S., Morris, J., Ono, K., Pavlovic, V., Pico, A.R., Vailaya, A., Wang, P.-L., Adler, A., Conklin, B.R., Hood, L., Kuiper, M., Sander, C., Schmulevich, I., Schwikowski, B., Warner, G.J., Ideker, T., Bader, G.D., 2007. Integration of biological networks and gene expression data using Cytoscape _ Nature Protocols, Nature Protocols. Nature Publishing Group. https://doi.org/10.1038/nprot.2007.324

Consortium, T.U., 2021. UniProt: the universal protein knowledgebase in 2021. Nucleic Acids Res. 49, D480–D489. https://doi.org/10.1093/nar/gkaa1100

Copeland, P.R., Fletcher, J.E., Carlson, B.A., Hatfield, D.L., Driscoll, D.M., 2000. A novel RNA binding protein, SBP2, is required for the translation of mammalian selenoprotein mRNAs. EMBO J. 19, 306–314. https://doi.org/10.1093/emboj/19.2.306

Csermely, P., Ágoston, V., Pongor, S., 2005. The efficiency of multi-target drugs: The network approach might help drug design. Trends Pharmacol. Sci. https://doi.org/10.1016/j.tips.2005.02.007

Du, Y., Hu, H., Qu, S., Wang, J., Hua, C., Zhang, J., Wei, P., He, X., Hao, J., Liu, P., Yang, F., Li, T., Wei, T., 2018. SIRT5 deacylates metabolism-related proteins and attenuates hepatic steatosis in ob/ob mice. EBioMedicine 36, 347–357. https://doi.org/10.1016/j.ebiom.2018.09.037

Fang, M., Guo, W.R., Park, Y., Kang, H.G., Zarbl, H., 2015. Enhancement of NAD+-dependent SIRT1 deacetylase activity by methylselenocysteine resets the circadian clock in carcinogen-treated mammary epithelial cells. Oncotarget 6, 42879–42891. https://doi.org/10.18632/oncotarget.6002

Ferguson, L.R., Karunasinghe, N., Zhu, S., Wang, A.H., 2012. Selenium and its role in the maintenance of genomic stability. Mutat. Res. 733, 100–110.

https://doi.org/10.1016/j.mrfmmm.2011.12.011

Franz, M., Rodriguez, H., Lopes, C., Zuberi, K., Montojo, J., Bader, G.D., Morris, Q., 2018. GeneMANIA update 2018. Nucleic Acids Res. 46, W60–W64. https://doi.org/10.1093/nar/gky311

Garrow, T.A., 1996. Purification, kinetic properties, and cDNA cloning of mammalian betaine-homocysteine methyltransferase *. J. Biol. Chem. 271, 22831–22838. https://doi.org/10.1074/jbc.271.37.22831

Garrow, T.A., Brenner, A.A., Whitehead, V.M., Chen, X.N., Duncan, R.G., Korenberg, J.R., Shane, B., 1993. Cloning of human cDNAs encoding mitochondrial and cytosolic serine hydroxymethyltransferases and chromosomal localization. J. Biol. Chem. 268, 11910–11916. https://doi.org/10.1016/s0021-9258(19)50286-1

Giacconi, R., Chiodi, L., Boccoli, G., Costarelli, L., Piacenza, F., Provinciali, M., Malavolta, M., 2021. Reduced levels of plasma selenium are associated with increased inflammation and cardiovascular disease in an Italian elderly population. Exp. Gerontol. 145, 111219. https://doi.org/https://doi.org/10.1016/j.exger.2020.111219

Greene, K.S., Lukey, M.J., Wang, X., Blank, B., Druso, J.E., Lin, M.C.J., Stalnecker, C.A., Zhang, C., Abril, Y.N., Erickson, J.W., Wilson, K.F., Lin, H., Weiss, R.S., Cerione, R.A., 2019. SIRT5 stabilizes mitochondrial glutaminase and supports breast cancer tumorigenesis. Proc. Natl. Acad. Sci. U. S. A. 116, 26625–26632. https://doi.org/10.1073/pnas.1911954116

Grosshans, H., Hurt, E., Simos, G., 2000. An aminoacylation-dependent nuclear tRNA export pathway in yeast. Genes Dev. 14, 830–840. https://doi.org/10.1101/gad.14.7.830

Guan, J., Jiang, X., Gai, J., Sun, X., Zhao, J., Li, J., Li, Y., Cheng, M., Du, T., Fu, L., Li, Q.,

2020. Sirtuin 5 regulates the proliferation, invasion, and migration of prostate cancer cells through acetyl-CoA acetyltransferase 1. J. Cell. Mol. Med. 24, 14039–14049. https://doi.org/10.1111/jcmm.16016

Haigis, M.C., Deng, C.X., Finley, L.W.S., Kim, H.S., Gius, D., 2012. SIRT3 is a mitochondrial tumor suppressor: A scientific tale that connects aberrant cellular ROS, the Warburg effect, and carcinogenesis. Cancer Res. 72, 2468–2472. https://doi.org/10.1158/0008-5472.CAN-11-3633

Hamosh, A., Scott, A.F., Amberger, J., Valle, D., McKusick, V.A., 2000. Online Mendelian Inheritance In Man (OMIM). Hum. Mutat. 15, 57–61. https://doi.org/https://doi.org/10.1002/(SICI)1098-1004(200001)15:1<57::AID-HUMU12>3.0.CO;2-G

Hershberger, K.A., Abraham, D.M., Martin, A.S., Mao, L., Liu, J., Gu, H., Locasale, J.W., Hirschey, M.D., 2017. Sirtuin 5 is required for mouse survival in response to cardiac pressure overload. J. Biol. Chem. 292, 19767–19781. https://doi.org/10.1074/jbc.M117.809897

Hu, Y., Spengler, M.L., Kuropatwinski, K.K., Comas, M., Jackson, M., Chernov, M. V., Gleiberman, A.S., Fedtsova, N., Rustum, Y.M., Gudkov, A. V., Antoch, M.P., 2011. Selenium is a modulator of circadian clock that protects mice from the toxicity of a chemotherapeutic drug via upregulation of the core clock protein, BMAL1. Oncotarget 2, 1279–1290. https://doi.org/10.18632/oncotarget.411

Jacobs, K.M., Pennington, J.D., Bisht, K.S., Aykin-Burns, N., Kim, H.-S., Mishra, M., Sun, L., Nguyen, P., Ahn, B.-H., Leclerc, J., Deng, C.-X., Spitz, D.R., Gius, D., 2008. SIRT3 interacts with the daf-16 homolog FOXO3a in the Mitochondria, as well as increases

FOXO3a Dependent Gene expression. Int. J. Biol. Sci. 4, 291–299. https://doi.org/10.7150/ijbs.4.291

Jain, M., Nilsson, R., Sharma, S., Madhusudhan, N., Kitami, T., Souza, A.L., Kafri, R., Kirschner, M.W., Clish, C.B., Mootha, V.K., 2012. Metabolite profiling identifies a key role for glycine in rapid cancer cell proliferation. Science 336, 1040–1044. https://doi.org/10.1126/science.1218595

Jintang, D., Yeyun, Z., Xiaoyang, S., Jiu, Y.J., Saba, K., Hong, J., Jungwoo, K., Jimin, W., Huyn, K.J., Hyun, C.B., Bin, H., Wei, C., Sheng, Z., A., C.R., Johan, A., Quan, H., Hening, L., Du, J., Zhou, Y., Su, X., Yu, J.J., Khan, S., Jiang, H., Kim, J.H., Woo, J., Kim, J.H., Choi, B.H., He, B., Chen, W., Zhang, S., Cerione, R.A., Auwerx, J., Hao, Q., Lin, H., 2011. Sirt5 is a NAD-dependent protein lysine demalonylase and desuccinylase. Science (80-.). 334, 806–809. https://doi.org/10.1126/science.1207861

Jung, M., Ilyashov, I., Park, Y., Donovan, F.X., Ramanagoudr-Bhojappa, R., Keahi, D., Durmaz, J.A., Choijilsuren, H.B., Goldfarb, A., Stein, M., Kim, J., White, R.R., Sridhar, S., Noonan, R., Wiley, T., Carroll, T.S., Lach, F.P., Auerbach, A.D., Miranda, I., Chandrasekharappa, S.C., Smogorzewska, A., 2022. ALDH9A1 Deficiency as a Source of Endogenous DNA Damage that Requires Repair by the Fanconi Anemia Pathway. BioRxiv 2022.05.26.493623. https://doi.org/10.1101/2022.05.26.493623

Kaisaki, P.J., Otto, G.W., McGouran, J.F., Toubal, A., Argoud, K., Waller-Evans, H., Finlay, C., Caldérari, S., Bihoreau, M.T., Kessler, B.M., Gauguier, D., Mott, R., 2014. Genetic control of differential acetylation in diabetic rats. PLoS One 9. https://doi.org/10.1371/journal.pone.0094555

Kim, D., Fiske, B.P., Birsoy, K., Freinkman, E., Kami, K., Possemato, R., Chudnovsky, Y.,

Michael, E., Chen, W.W., Cantor, J.R., Shelton, L.M., Dan, Y., Kwon, M., Ramkissoon, S.H., Ligon, K.L., Woo, S., Snuderl, M., Heiden, M.G. Vander, David, M., 2015. SHMT2 drives glioma cell survival in the tumor microenvironment but imposes a dependence on glycine clearance. Nature 520, 363–367. https://doi.org/10.1038/nature14363.SHMT2

Kim, S.H., Kim, B.K., Park, S.K., 2018. Selenocysteine mimics the effect of dietary restriction on lifespan via SKN-1 and retards age-associated pathophysiological changes in Caenorhabditis elegans. Mol. Med. Rep. 18, 5389–5398. https://doi.org/10.3892/mmr.2018.9590

Krynetski, E.Y., Krynetskaia, N.F., Bianchi, M.E., Evans, W.E., 2003. A nuclear protein complex containing high mobility group proteins B1 and B2, heat shock cognate protein 70, ERp60, and glyceraldehyde-3-phosphate dehydrogenase is involved in the cytotoxic response to DNA modified by incorporation of anticancer nucleoside. Cancer Res. 63, 100–106.

Lombard, D.B., Alt, F.W., Cheng, H.-L., Bunkenborg, J., Streeper, R.S., Mostoslavsky, R., Kim, J., Yancopoulos, G., Valenzuela, D., Murphy, A., Yang, Y., Chen, Y., Hirschey, M.D., Bronson, R.T., Haigis, M., Guarente, L.P., Farese, R. V., Weissman, S., Verdin, E., Schwer, B., 2007. Mammalian Sir2 Homolog SIRT3 Regulates Global Mitochondrial Lysine Acetylation. Mol. Cell. Biol. 27, 8807–8814. https://doi.org/10.1128/mcb.01636-07

Lopes, C.T., Franz, M., Kazi, F., Donaldson, S.L., Morris, Q., Bader, G.D., 2010. Cytoscape Web: an interactive web-based network browser. Bioinformatics 26, 2347–2348. https://doi.org/10.1093/bioinformatics/btq430

Matsushita, N., Yonashiro, R., Ogata, Y., Sugiura, A., Nagashima, S., Fukuda, T., Inatome, R., Yanagi, S., 2011. Distinct regulation of mitochondrial localization and stability of two

human Sirt5 isoforms. Genes to Cells 16, 190–202. https://doi.org/https://doi.org/10.1111/j.1365-2443.2010.01475.x

McDonnell, E., Peterson, B.S., Bomze, H.M., Hirschey, M.D., 2015. SIRT3 regulates progression and development of diseases of aging. Trends Endocrinol. Metab. 26, 486–492. https://doi.org/10.1016/j.tem.2015.06.001

Nahálková, J., 2022. Focus on Molecular Functions of Anti-Aging Deacetylase SIRT3. Biochem. 87, 21–34. https://doi.org/10.1134/S0006297922010035

Nakagawa, T., Lomb, D.J., Haigis, M.C., Guarente, L., 2009. SIRT5 Deacetylates Carbamoyl Phosphate Synthetase 1 and Regulates the Urea Cycle. Cell 137, 560–570. https://doi.org/https://doi.org/10.1016/j.cell.2009.02.026

Nishida, Y., Rardin, M.J., Carrico, C., He, W., Sahu, A.K., Gut, P., Najjar, R., Fitch, M., Hellerstein, M., Gibson, B.W., Verdin, E., 2015. SIRT5 Regulates both Cytosolic and Mitochondrial Protein Malonylation with Glycolysis as a Major Target. Mol. Cell 59, 321–332. https://doi.org/10.1016/j.molcel.2015.05.022

Nolan, G.P., 2007. What's wrong with drug screening today. Nat. Chem. Biol. https://doi.org/10.1038/nchembio0407-187

Ogura, M., Nakamura, Y., Tanaka, D., XT, Z., Fujita, Y., Obara, A., Hamasaki, A., Hosokawa, M., Inagaki, N., 2010. Overexpression of SIRT5 confirms its involvement in deacetylation and activation of carbamoyl phosphate synthetase 1. Biochem. Biophys. Res. Commun. 393, 73–78. https://doi.org/10.1016/j.bbrc.2010.01.081

Oti, M., 2006. Predicting disease genes using protein-protein interactions. J. Med. Genet. 43, 691–698. https://doi.org/10.1136/jmg.2006.041376

Paone, A., Marani, M., Fiascarelli, A., Rinaldo, S., Giardina, G., Contestabile, R., Paiardini, A.,

Cutruzzolà, F., 2014. SHMT1 knockdown induces apoptosis in lung cancer cells by causing uracil misincorporation. Cell Death Dis. 5, 1–11. https://doi.org/10.1038/cddis.2014.482

Park, J., Chen, Y., Tishkoff, D.X., Peng, C., Tan, M., Dai, L., Xie, Z., Zhang, Y., Zwaans, B.M.M.M., Skinner, M.E., Lombard, D.B., Zhao, Y., 2013. SIRT5-Mediated Lysine Desuccinylation Impacts Diverse Metabolic Pathways. Mol. Cell 50, 919–930. https://doi.org/10.1016/j.molcel.2013.06.001

Peng, C., Lu, Z., Xie, Z., Cheng, Z., Chen, Y., Tan, M., Luo, H., Zhang, Y., He, W., Yang, K., Zwaans, B.M.M.M., Tishkoff, D., Ho, L., Lombard, D., He, T.-C.C., Dai, J., Verdin, E., Ye, Y., Zhao, Y., 2011. The first identification of lysine malonylation substrates and its regulatory enzyme. Mol. Cell. Proteomics 10, M111.012658. https://doi.org/10.1074/mcp.M111.012658

Pillai, V.B., Sundaresan, N.R., Kim, G., Gupta, M.M.P.M., Rajamohan, S.B., Pillai, J.B., Samant, S., Ravindra, P. V., Isbatan, A., Gupta, M.M.P.M., 2010. Exogenous NAD blocks cardiac hypertrophic response via activation of the SIRT3-LKB1-AMP-activated kinase pathway. J. Biol. Chem. 285, 3133–3144. https://doi.org/10.1074/jbc.M109.077271

Pourhaghighi, R., Ash, P.E.A., Phanse, S., Goebels, F., Hu, L.Z.M., Chen, S., Zhang, Y., Wierbowski, S.D., Boudeau, S., Moutaoufik, M.T., Malty, R.H., Malolepsza, E., Tsafou, K., Nathan, A., Cromar, G., Guo, H., Abdullatif, A. Al, Apicco, D.J., Becker, L.A., Gitler, A.D., Pulst, S.M., Youssef, A., Hekman, R., Havugimana, P.C., White, C.A., Blum, B.C., Ratti, A., Bryant, C.D., Parkinson, J., Lage, K., Babu, M., Yu, H., Bader, G.D., Wolozin, B., Emili, A., 2020. BraInMap Elucidates the Macromolecular Connectivity Landscape of Mammalian Brain. Cell Syst. 10, 333-350.e14. https://doi.org/10.1016/j.cels.2020.03.003

Rardin, M.J., He, W., Nishida, Y., Newman, J.C., Carrico, C., Danielson, S.R., Guo, A., Gut, P.,

Sahu, A.K., Li, B., Uppala, R., Fitch, M., Riiff, T., Zhu, L., Zhou, J., Mulhern, D., Stevens, R.D., Ilkayeva, O.R., Newgard, C.B., Jacobson, M.P., Hellerstein, M., Goetzman, E.S., Gibson, B.W., Verdin, E., 2013. SIRT5 Regulates the Mitochondrial Lysine Succinylome and Metabolic Networks. Cell Metab. 18, 920–933. https://doi.org/https://doi.org/10.1016/j.cmet.2013.11.013

Sadhukhan, S., Liu, X., Ryu, D., Nelson, O.D., Stupinski, J.A., Li, Z., Chen, W., Zhang, S., Weiss, R.S., Locasale, J.W., Auwerx, J., Lin, H., 2016. Metabolomics-assisted proteomics identifies succinylation and SIRT5 as important regulators of cardiac function. Proc. Natl. Acad. Sci. U. S. A. 113, 4320–4325. https://doi.org/10.1073/pnas.1519858113

Salonen, J., Alfthan, G., Huttunen, J., Pikkarainen, J., Puska, P., 1982. Association between cardiovascular death, myocardial infarction, and serum selenium in a matched-pair longitudinal study. Lancet 320, 175–179. https://doi.org/10.1016/S0140-6736(82)91028-5

Schirch, L., 1982. Serine hydroxymethyltransferase. Adv. Enzymol. Relat. Areas Mol. Biol. 53, 83–112. https://doi.org/10.1002/9780470122983.ch3

Schlicker, C., Gertz, M., Papatheodorou, P., Kachholz, B., Becker, C.F.W., Steegborn, C., 2008. Substrates and Regulation Mechanisms for the Human Mitochondrial Sirtuins Sirt3 and Sirt5. J. Mol. Biol. 382, 790–801. https://doi.org/https://doi.org/10.1016/j.jmb.2008.07.048

Seale, L.A., Hashimoto, A.C., Kurokawa, S., Gilman, C.L., Seyedali, A., Bellinger, F.P., Raman, A. V., Berry, M.J., 2012. Disruption of the Selenocysteine Lyase-Mediated Selenium Recycling Pathway Leads to Metabolic Syndrome in Mice. Mol. Cell. Biol. 32, 4141–4154. https://doi.org/10.1128/mcb.00293-12

Suadicani, P., Hein, H.O., Gyntelberg, F., 1992. Serum selenium concentration and risk of ischaemic heart disease in a prospective cohort study of 3000 males. Atherosclerosis 96,

33–42. https://doi.org/10.1016/0021-9150(92)90035-F

Tan, M., Peng, C., Anderson, K.A.A.A., Chhoy, P., Xie, Z., Dai, L., Park, J., Chen, Y., Huang, H., Zhang, Y., Ro, J., Wagner, G.R.R.R., Green, M.F.F.F., Madsen, A.S.S.S., Schmiesing, J., Peterson, B.S.S.S., Xu, G., Ilkayeva, O.R.R.R., Muehlbauer, M.J.J.J., Braulke, T., Mühlhausen, C., Backos, D.S.S.S., Olsen, C.A.A.A., McGuire, P.J.J.J., Pletcher, S.D.D.D., Lombard, D.B.B.B., Hirschey, M.D.D.D., Zhao, Y., 2014. Lysine glutarylation is a protein posttranslational modification regulated by SIRT5. Cell Metab. 19, 605–617. https://doi.org/10.1016/j.cmet.2014.03.014

Teng, Y.W., Mehedint, M.G., Garrow, T.A., Zeisel, S.H., 2011. Deletion of betaine-homocysteine S-methyltransferase in mice perturbs choline and 1-carbon metabolism, resulting in fatty liver and hepatocellular carcinomas. J. Biol. Chem. 286, 36258–36267. https://doi.org/10.1074/jbc.M111.265348

Tomita, T., 2017. Aberrant proteolytic processing and therapeutic strategies in Alzheimer's disease. Adv. Biol. Regul. 64, 33–38. https://doi.org/10.1016/j.jbior.2017.01.001

Tong, W., Ju, L., Qiu, M., Xie, Q., Chen, Y., Shen, W., Sun, W., Wang, W., Tian, J., 2016. Liraglutide ameliorates non-alcoholic fatty liver disease by enhancing mitochondrial architecture and promoting autophagy through the SIRT1/SIRT3–FOXO3a pathway. Hepatol. Res. 46, 933–943. https://doi.org/10.1111/hepr.12634

Torres, C.M., Biran, A., Burney, M.J., Patel, H., Henser-Brownhill, T., Cohen, A.H.S., Li, Y., Ben-Hamo, R., Nye, E., Spencer-Dene, B., Chakravarty, P., Efroni, S., Matthews, N., Misteli, T., Meshorer, E., Scaffidi, P., 2016. The linker histone H1.0 generates epigenetic and functional intratumor heterogeneity. Science (80-.). 353, 1–28. https://doi.org/10.1126/science.aaf1644

Uthus, E.O., Ross, S.A., Davis, C.D., 2006. Differential effects of dietary selenium (Se) and folate on methyl metabolism in liver and colon of rats. Biol. Trace Elem. Res. 109, 201–214. https://doi.org/10.1385/BTER:109:3:201

Wang, L., Wang, W.-Y., Cao, L.-P., 2015. SIRT3 inhibits cell proliferation in human gastric cancer through down-regulation of Notch-1. Int. J. Clin. Exp. Med. 8, 5263–71.

Wang, Y.C., Lin, W.L., Lin, Y.J., Tang, F.Y., Chen, Y.M., Chiang, E.P.I., 2014. A novel role of the tumor suppressor GNMT in cellular defense against DNA damage. Int. J. Cancer 134, 799–810. https://doi.org/10.1002/ijc.28420

Wang, Y.Q., Wang, H.L., Xu, J., Tan, J., Fu, L.N., Wang, J.L., Zou, T.H., Sun, D.F., Gao, Q.Y., Chen, Y.X., Fang, J.Y., 2018. Sirtuin5 contributes to colorectal carcinogenesis by enhancing glutaminolysis in a deglutarylation-dependent manner. Nat. Commun. 9, 1–15. https://doi.org/10.1038/s41467-018-02951-4

Warde-Farley, D., Donaldson, S.L., Comes, O., Zuberi, K., Badrawi, R., Chao, P., Franz, M., Grouios, C., Kazi, F., Lopes, C.T., Maitland, A., Mostafavi, S., Montojo, J., Shao, Q., Wright, G., Bader, G.D., Morris, Q., 2010. The GeneMANIA prediction server: Biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res. 38, 214–220. https://doi.org/10.1093/nar/gkq537

Wei, Z., Song, J., Wang, G., Cui, X., Zheng, J., Tang, Y., Chen, X., Li, J., Cui, L., Liu, C.Y., Yu, W., 2018. Deacetylation of serine hydroxymethyl-transferase 2 by SIRT3 promotes colorectal carcinogenesis. Nat. Commun. 9. https://doi.org/10.1038/s41467-018-06812-y

Yan, D., Franzini, A., Pomicter, A.D., Halverson, B.J., Antelope, O., Mason, C.C., Ahmann, J.M., Senina, A. V., Vellore, N.A., Jones, C.L., Zabriskie, M.S., Than, H., Xiao, M.J., van Scoyk, A., Patel, A.B., Clair, P.M., Heaton, W.L., Owen, S.C., Andersen, J.L., Egbert,

C.M., Reisz, J.A., D'Alessandro, A., Cox, J.E., Gantz, K.C., Redwine, H.M., Iyer, S.M., Khorashad, J.S., Rajabi, N., Olsen, C.A., O'Hare, T., Deininger, M.W., 2021. SIRT5 Is a Druggable Metabolic Vulnerability in Acute Myeloid Leukemia. Cancer Discov. 2, 266–287. https://doi.org/10.1158/2643-3230.BCD-20-0168

Yang, X., Wang, Z., Li, X., Liu, B., Liu, M., Liu, L., Chen, S., Ren, M., Wang, Y., Yu, M., Wang, B., Zou, J., Zhu, W.G., Yin, Y., Gu, W., Luo, J., 2018. Shmt2 desuccinylation by SIRT5 drives cancer cell proliferation. Cancer Res. 78, 372–386. https://doi.org/10.1158/0008-5472.CAN-17-1912

Yeung, F., Hoberg, J.E., Ramsey, C.S., Keller, M.D., Jones, D.R., Frye, R.A., Mayo, M.W., 2004. Modulation of NF-κB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J. 23, 2369–2380. https://doi.org/10.1038/sj.emboj.7600244

Yu, J., Sadhukhan, S., Noriega, L.G., Moullan, N., He, B., Weiss, R.S., Lin, H., Schoonjans, K., Auwerx, J., 2013. Metabolic characterization of a Sirt5 deficient mouse model. Sci. Rep. 3, 1–7. https://doi.org/10.1038/srep02806

Yu, W., Gao, B., Li, N., Wang, J., Qiu, C., Zhang, G., Liu, M., Zhang, R., Li, C., Ji, G., Zhang, Y., 2017. Sirt3 deficiency exacerbates diabetic cardiac dysfunction: Role of Foxo3A-Parkin-mediated mitophagy. Biochim. Biophys. Acta - Mol. Basis Dis. 1863, 1973–1983. https://doi.org/10.1016/j.bbadis.2016.10.021

Zeng, Y., Zhang, J., Xu, M., Chen, F., Zi, R., Yue, J., Zhang, Y., Chen, N., Chin, Y.E., 2021. Roles of mitochondrial serine hydroxymethyltransferase 2 (SHMT2) in human carcinogenesis. J. Cancer 12, 5888–5894. https://doi.org/10.7150/jca.60170

Zuberi, K., Franz, M., Rodriguez, H., Montojo, J., Lopes, C.T., Bader, G.D., Morris, Q., 2013. GeneMANIA prediction server 2013 update. Nucleic Acids Res. 41, 115–122.

https://doi.org/10.1093/nar/gkt533

Fig. 1 The results of the pathway enrichment and gene function prediction analysis of the interaction network of lysine malonylated substrates of SIRT5. The network was constructed by GeneMania (3.5.2) application run under Cytoscape (3.9.1) environment utilizing the *H. sapiens* database (29-04-2021) and including all types of interaction networks. The clusters of the most important pathways ETE, AADM, and SAM, and their overlapping and unique nodes are visualized in different colors. The most significantly enriched general Metabolism pathway is not marked and discussed.

Fig. 2 A model of the interactions between SIRT3, SIRT5, and Selenoamino acid metabolism. The presence of mitochondrial (m), cytoplasmic (c), and nuclear (n) SIRT5 is supported by the subcellular localization of the lysine malonylated substrates of SIRT5 and scientific reports by other authors. Selenocysteine metabolism and SIRT5 functions are linked to hepatic steatosis (HS) pathology. Mice lacking selenocysteine lyase (ScLy) develop metabolic syndrome symptoms including HS, which is attenuated in the obesity ob/ob mice model by overexpression of the SIRT5. Selenocysteine mimics the effect of calorie restriction (CR) similar to exercise and increases the NAD+/NADH ratio, which further stimulates the activity of SIRT5 and other sirtuins. Selenocysteine also decreases the effect of one of the hallmarks of Alzheimer´s disease Aβ and the associated toxicity strongly connected to aging. SIRT5 functions as a tumor promoter are known, however, its lysine demalonylation of the multiple substrates with tumor promoter and suppressor activities suggests dual tumor suppressor/oncogene functions similarly to the directly interacting SIRT3. A link is indicated between the selenium levels, expression of SIRT3, SIRT5, and cardiac disease, which is supported by several experimental pieces of evidence. The maintenance of NAD+ levels in the heart is also important for preventing cardiac failure. Methylselenocystein (MSC) affects the circadian cycle by restoring the NAD^+/NADH ratio and the sirtuin activities. Stimulated SIRT1 expression deacetylates AcBMAL1, decreases its binding to the E-box element of the promoter of PER2, and rises its circadian transcription. The regulation of the circadian cycle also occurs through the SIRT3-FOXO3-CLOCK pathway, where FOXO3 is the crucial regulator.

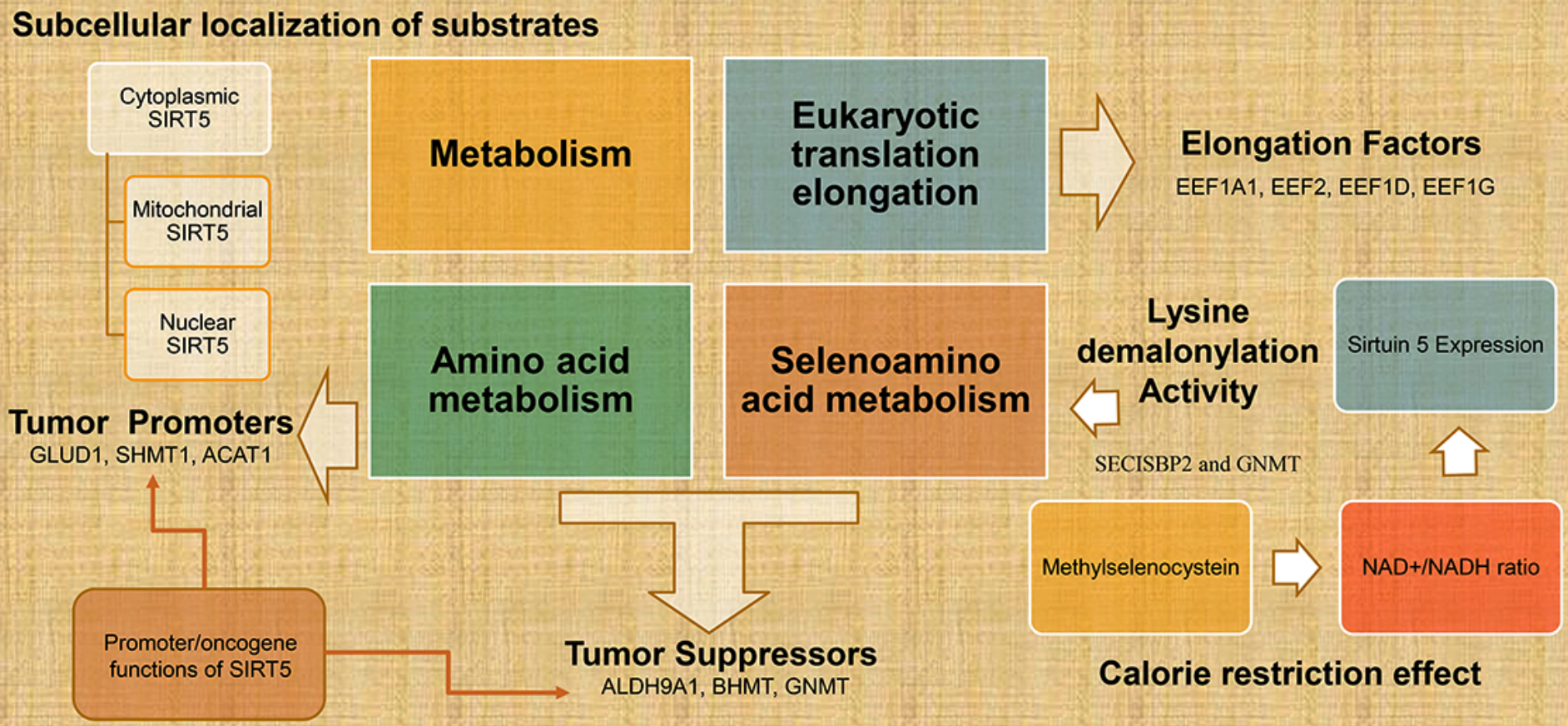
Tab. 1 The results of the pathway enrichment and gene function prediction analysis of the interaction network of lysine malonylated substrates of SIRT5. The network was constructed by GeneMania (3.5.2) application run under Cytoscape (3.9.1) environment utilizing the *H. sapiens* database (29-04-2021) and including all types of interaction networks. Scores (weights) express the predictive value, that GeneMania assigns to the pathways, and how well they correspond to the query dataset compared to the non-query. The results of the categories Reactome and Consolidated Pathways are shown.

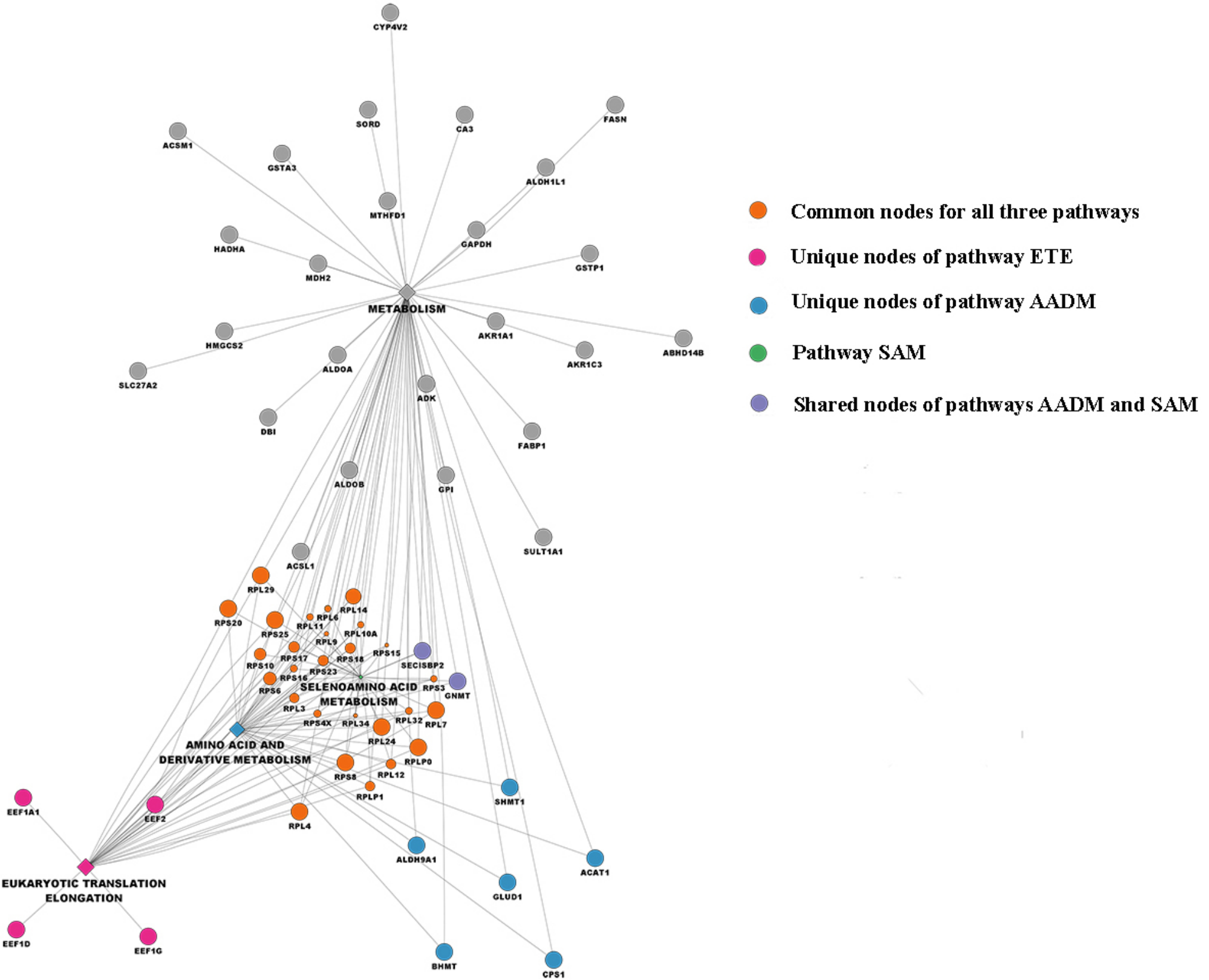
Tab. S1 The full results of the pathway enrichment and gene function prediction analysis of the interaction network of lysine malonylated substrates of SIRT5. The table includes the input protein list (column Gene 7-76).

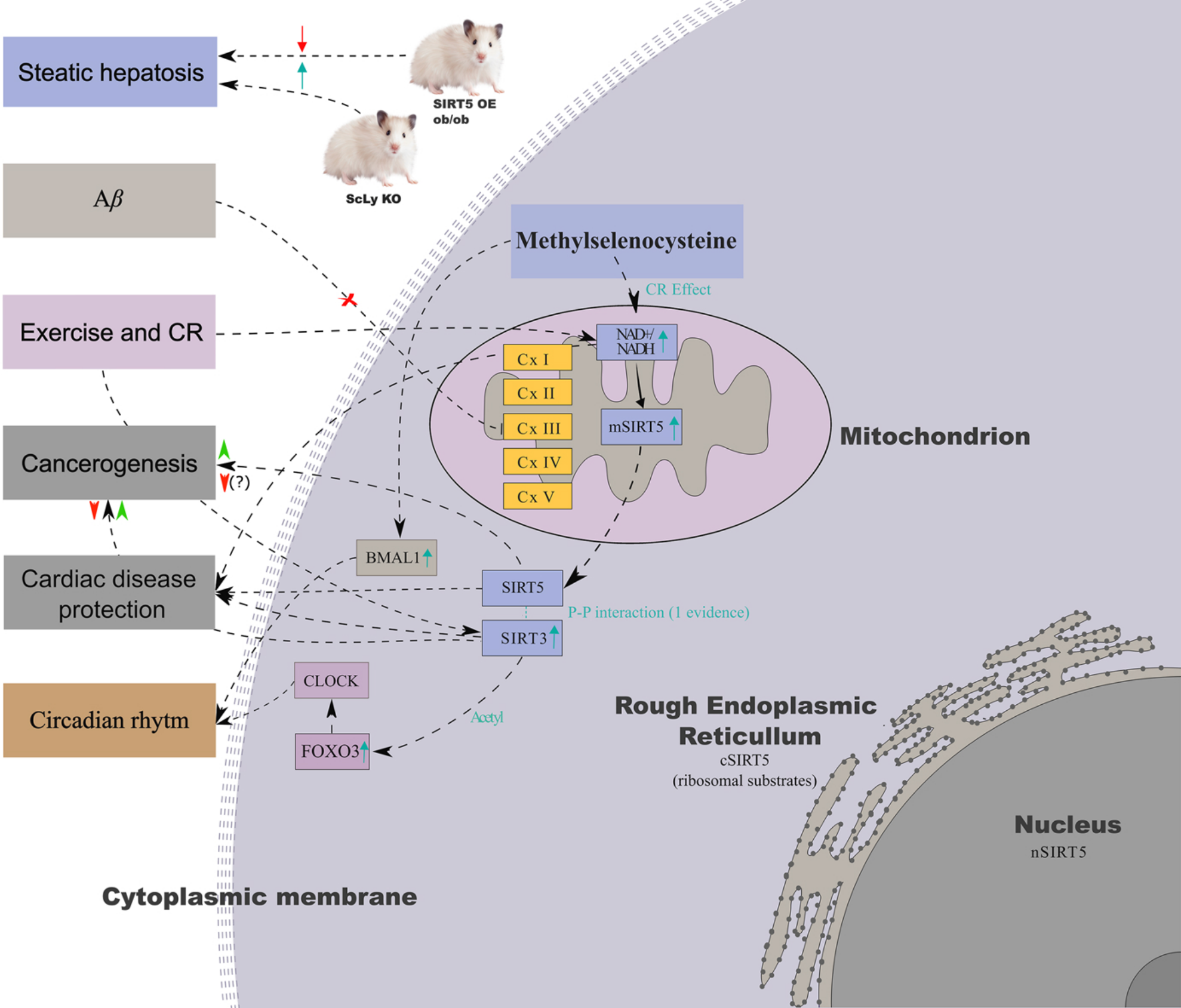
Highlights

- SIRT5 lysine demalonylates elongation factors participating in proteosynthesis
- Tumor suppressor/promoter functions of lysine malonylated substrates of SIRT5
- Cancer-related functions of substrates are associated with the amino acid metabolism
- SIRT5 regulates the selenoamino acid metabolism and selenium controls SIRT5 expression

The interaction network analysis of lysine demalonylation substrates of SIRT5




CYP4V2
SORD
CA3
FASN
ACSM1
GSTA3
ALDH1L1
MTHFD1
HADHA
GAPDH
GSTP1
MDH2
METABOLISM
AKR1A1
HMGCS2
ABHD14B
AKR1C3
ALDOA
SLC27A2
ADK
DBI
FABP1
ALDOB
GPI
SULT1A1
ACSL1
RPL29
RPL14
RPS20
RPL6
RPS25
RPL11
RPL10A
RPL9
RPS15
RPS17
RPS18
RPS10
SECISBP2
RPS23
RPS16
RPS6
SELENOAMINO ACID METABOLISM
RPS3
GNMT
RPL3
RPS4X
RPL34
RPL32
RPL7
RPL24
AMINO ACID AND DERIVATIVE METABOLISM
RPLP0
RPS8
RPL12
RPLP1
SHMT1
EEF1A1
EEF2
RPL4
ALDH9A1
ACAT1
EUKARYOTIC TRANSLATION ELONGATION
GLUD1
EEF1D
EEF1G
BHMT
CPS1
Common nodes for all three pathways
Unique nodes of pathway ETE
Unique nodes of pathway AADM
Pathway SAM
Shared nodes of pathways AADM and SAM


Steatic hepatosis
SIRT5 OE
ob/ob
ScLy KO
Aβ
Methylselenocysteine
CR Effect
Exercise and CR
NAD+/
NADH
Cx I
Cx II
Cx III
Cx IV
Cx V
mSIRT5
Mitochondrion
Cancerogenesis
(?)
BMAL1
Cardiac disease
protection
SIRT5
P-P interaction (1 evidence)
SIRT3
CLOCK
Circadian rhytm
Acetyl
FOXO3
Rough Endoplasmic
Reticullum
cSIRT5
(ribosomal substrates)
Nucleus
nSIRT5
Cytoplasmic membrane

Type of the database	Pathway	Score
		11,35
Reactome		
	Metabolism ID Release 72 1430728	3,23
	Eukaryotic Translation Elongation R-HSA-156842.2	2,13
	Amino acid and derivative metabolism R-HSA-71291.6	2,07
	Selenoamino acid metabolism 72 2408522	0,9
	Cellular responses to stress ID Release 72 2262752	0,89
	Cellular responses to external stimuli R-HSA-8953897.4	0,86
	Translation R-HSA-72766.4	0,69
	Peptide chain elongation R-HSA-156902.2	0,39
	Selenocysteine synthesis R-HSA-2408557.2	0,2
		8,22
Consolidated Pathways		
	Eukaryotic translation elongation React 1477.2	2,45
	Translation React 1014.4	1,9
	Metabolic pathways KEGG hsa01100	1,65
	Metabolism React 111217.2	1,64
	Peptide chain elongation React 1404.2	0,53
	SRP-dependent co-translational protein targeting membrane React 115902.1	0,06