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Emerging considerations on mitochondrial and cytosolic metabolic features in SDH-deficient cancer cells

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Dear Editor,

Cytosolic and mitochondrial NADH to NAD^+ ratios are in equilibrium *via* redox shuttles. The shuttle involving subcellular aspartate aminotransferase and malate dehydrogenase isoforms with key roles of mitochondrial inner membrane SLC25A13 and SLC25A11 is depicted in Fig. 1.

Succinate Dehydrogenase (SDH) is a mitochondrial enzyme composed of 4 protein subunits: two anchored in the inner membrane (SDHC and SDHD) and two catalytic subunits protruding in the matrix (SDHA and SDHB) [1]. SDH catalyzes both the oxidation of succinate into fumarate (Krebs cycle) and the complex II activity which transfers electrons released during succinate oxidation to mitochondrial electron transport chain *via* coenzyme Q. Heterozygous pathogenic variants in one of the *SDHx genes* predispose to familial pheochromocytoma and/or paraganglioma, tumors developing from chromaffin cells of adrenals and extra-adrenal paraganglia, respectively [2]. Complex I activity is preserved under loss of complex II activity in murine immortalized $Sdhb^{-/-}$ chromaffin cells [3] and human paragangliomas *SDHx* mutated cells [4]. Active complex I would help chromaffin cells to tolerate the absence of SDH far better than other cell types such as murine fibroblasts or kidney cells which exhibit dual loss of complex I and II activities when *Sdhb* is invalidated [5]. However, if O_2 consumption rates in $Sdhb^{-/-}$ chromaffin cells may be normal [3], ATP production at the complex V level appears to be reduced.

There are limits in proposing consensus mechanisms for all SDH deficient cells as a result of cell-specific effects of SDH deficiency on mitochondrial bioenergetics and of *SDHx* germline mutations on tumoral phenotypes of patients [6,7]. The inability of murine models of *Sdhx* genetic inactivation to spontaneously generate paragangliomas is another limitation [6,7]. Nevertheless, Krebs'cycle metabolic features are consistently observed under loss of SDH activity in chromaffin cells. They include succinate yield from α-ketoglutarate essentially from glutamine rather than pyruvate (75% -80% succinate derives from glutamine) [3,5], and handling of pyruvate mainly by PC and mASAT to fuel aspartate synthesis [5] (Fig. 2). Aspartate can join cytosolic anabolic pathways, such as protein and nucleotide biosynthesis, *via* the citrin transporter encoded by *SLC25A13* (Fig. 2). This is consistent with the metabolic reprogramming of mAST and citrin (SLC25A13) highlighted by Fig. 2. As observed in citrin deficiency [8], the mitochondrial/cytosolic NADH redox shuttle might be altered in SDH deficiency as a consequence of impaired importation of malate back to mitochondria. Indeed, mitochondrial succinate which accumulates in cells defective for SDH may be exported in the cytosol *via* the transporter SLC25A10 (dicarboxylate carrier) but can also behave as an inhibitor of the mitochondrial malate 2oxo-glutarate antiport [9] encoded by *SLC25A11* (Fig. 2). To explain the accumulation of NADH observed in the cytosol of cells defective for SDH [3,5], we hypothetize that succinate-induced SLC25A11 inhibition causes a disruption of the NADH redox shuttle (Fig. 2).

The altered redox state (Fig. 2) might promote tumor development by stimulating anabolic pathways such as fatty acid synthase which currently emerges as a booster of tumoral initiation [11]. Interestingly, inactivating germline mutations of *SLC25A11* also promote tumor development and are responsible for hereditary paragangliomas [12], suggesting a possible protective role of this protein against chromaffin tumoral development.

Last but not least, mitochondrial partition of pyruvate between dehydrogenation and carboxylation in SDH deficiency mainly fuel pyruvate carboxylation to oxaloacetate [5,13] (Fig. 2). Contribution of

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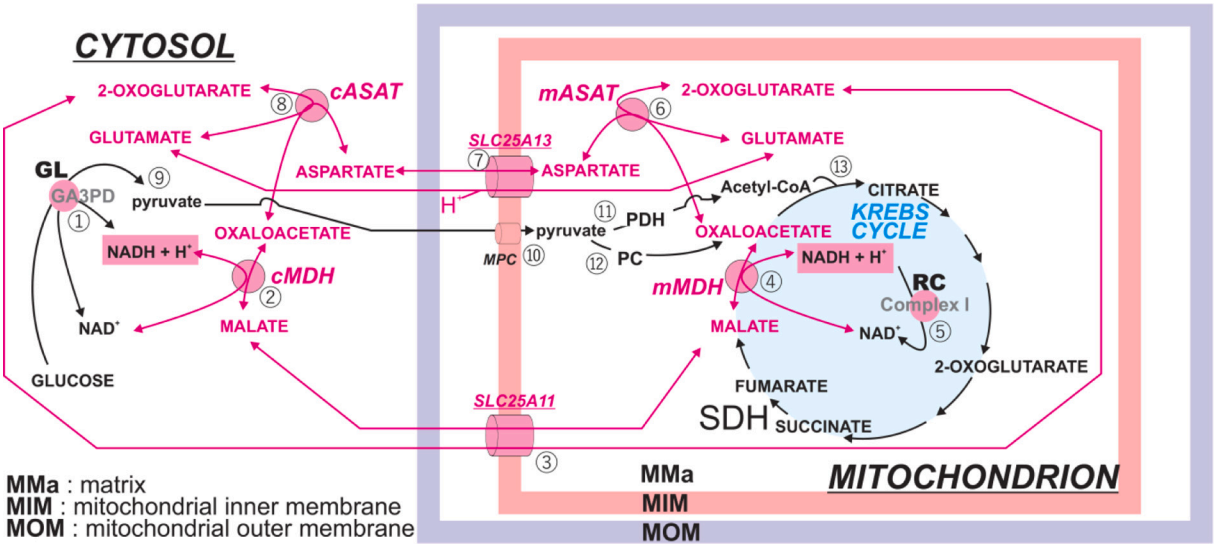

Fig. 1. Cytosolic-mitochondrial NADH redox shuttle involving SLC25A13 and SLC25A11.
NADH produced during glycolysis (GL) by glyceraldehyde-3-phosphate dehydrogenase (GA3PD) may be transferred from cytosol to mitochondria. This transfer involves cytosolic malate dehydrogenase (cMDH) ②, and mitochondrial inner membrane SCL25A11 (malate/2-oxoglutarate antiport) ③. Mitochondrial malate dehydrogenase (mMDH) ④ restitutes back NADH further oxidized by respiratory chain (RC) complex I ⑤. Mitochondrial aspartate aminotransferase (mASAT) ⑥ converts oxaloacetate to aspartate by transamination with glutamate. Mitochondrial aspartate is exchanged by SLC25A13 (also referred to as citrin) ⑦ with cytosolic glutamate produced by cytosolic aspartate aminotransferase (cASAT) ⑧. Successive steps ②, ③, ④, ⑥, ⑦ and ⑧ constitute the redox shuttle (in pink). Glycolytic pyruvate ⑨ may enter mitochondria *via* the mitochondrial pyruvate carrier (MPC) ⑩). It may give rise to acetyl-CoA ⑪ and oxaloacetate ⑫ (the substrates of citrate synthase ⑬) through actions of pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC), respectively.

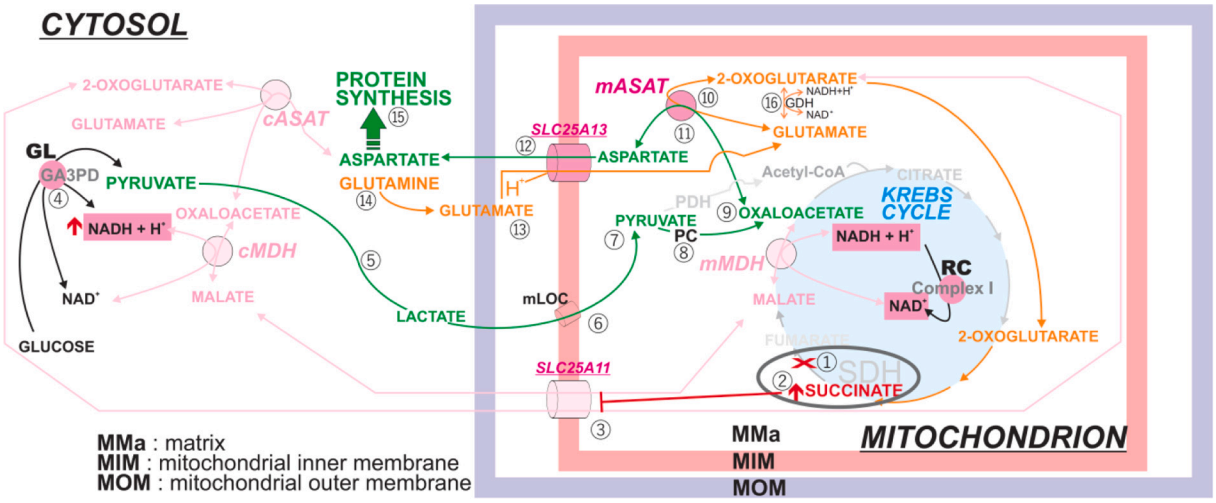

Fig. 2. Metabolic reprogramming in SDH-deficient cells.
Deficient SDH activity dramatically increases mitochondrial succinate ②, a known inhibitor of SLC25A11 ③. As a result, the NADH redox cycle (illustrated in Fig. 1) is disrupted, cytosolic NADH④, and lactate to pyruvate ratio ⑤ are raised up. Lactate fuelling of intramitochondrial pyruvate is proposed to involve mitochondrial lactate oxidation complex (mLOC) ⑥ described by Brooks [10]. Mitochondrial pyruvate ⑦ is converted by PC ⑧ to oxaloacetate ⑨ which may feed production by mASAT ⑩ of aspartate ⑪. Aspartate transsfer to cytosol occurs by citrin-catalyzed exchange ⑫ with glutamate ⑬. Glutamate is fuelled from glutamine by cytosolic and mitochondrial inner membrane glutaminases ⑭. Cytosolic aspartate contributes to protein synthesis ⑮.
Note that the oxidation of glutamate to 2-oxoglutarate by glutamate dehydrogenase (GDH) ⑯ bypasses mASAT, and dissociates succinate yield (in orange) from aspartate synthesis (in green).

PDH to the Krebs'cycle is then lowered. Maintenance of intra-mitochondrial NADH to support complex I activity should rest on other NAD^+ – dependent dehydrogenases involved in fatty acid and amino acid oxidations. When mitochondrial NADH to NAD^+ ratio becomes low, NAD^+-dependent glutamate dehydrogenase might act as a local "redoxstat", subtracting glutamate from mASAT reaction (Fig. 2, step ⑯). As a result, the exchange of aspartate with glutamine-derived glutamate would be reduced. Concomitantly, the availability of cytosolic glutamine for the reductive carboxylation pathway might be promoted as described in mitochondrial dysfunction [14].

The glutamine reductive carboxylation pathway enables supply from glutamine of cytosolic oxaloacetate to fuel cMDH reaction [14] which, by mitigating the high cytosolic NADH observed in SDH deficient cells, would alleviate the redox brake on glycolysis. Therein, fluctuations in mitochondrial NADH/NAD^+ ratio, which is regulated by complex I activity and regulates GDH, might induce fluctuations in partitioning glutamine metabolism between mitochondrial succinate synthesis (coupled to aspartate synthesis, see Fig. 2) and cytosolic reductive

carboxylation (coupled to glycolysis, see [14]). Through these mechanisms, complex I activity might increase the metabolic flexibility of SDH deficient cells.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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