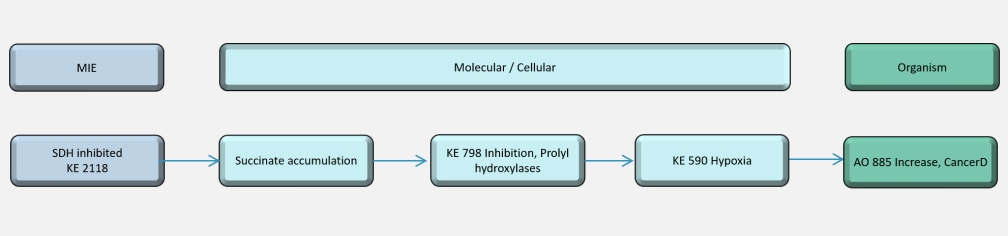
AOP ID and Title:

AOP 546: Succinate dehydrogenase inactivation leads to cancer through hypoxic-like mechanisms
Short Title: SDH inhibition, prolyl hydroxylase inhibition, pseudohypoxia

Graphical Representation



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Abstract

Succinate dehydrogenase (SDH) is a key enzymatic complex involved in two interconnected metabolic processes for energy production: the transfer of electrons in the mitochondrial respiratory chain and the oxidation of succinate to fumarate in the Krebs cycle. In humans, inherited SDH deficiencies may cause major pathologies including cancers. The cellular and molecular mechanisms related to genetic SDH inactivation have been well described in neuroendocrine tumors, in which it induces an oxidative stress, a pseudohypoxic phenotype, a metabolic, epigenetic and transcriptomic remodeling, and alterations in the migration and invasion capacities of cancer cells, in connection with the accumulation of succinate, an oncometabolite, substrate of the SDH. SDH complex is the molecular target of Succinate Dehydrogenase Inhibitors (SDHi), a family of pesticides widely used to limit the proliferation of pathogenic fungi. This AOP aims to describe the relationship between SDH inactivation and cancer development.

AOP Development Strategy

Context

Succinate dehydrogenase (SDH) is a key enzyme of mitochondria, organelles that play a crucial role in the production of energy, the metabolic and calcium homeostasis, the control of apoptosis, and the production of reactive oxygen species. SDH is involved in two interconnected metabolic processes for energy production: 1) cellular respiration, where it allows the transfer of electrons to ubiquinone as complex II of the mitochondrial respiratory chain, and 2) the Krebs cycle, where it catalyzes the oxidation of succinate to fumarate.

Numerous studies show that a complete inactivation of SDH caused by a first constitutional mutation associated with a second somatic mutation, leads to cancerous pathologies in young adults, including particularly aggressive forms of cancer such as paragangliomas (neuroendocrine tumors of the head and neck, thorax, abdomen and pelvis), pheochromocytomas (tumors of the adrenal medulla), renal cancers and gastrointestinal stromal tumors. The cellular and molecular mechanisms related to the genetic inactivation of SDH have been well described in neuroendocrine tumors, where it induces an oxidative stress, a pseudohypoxia phenotype, a metabolic, epigenetic and transcriptional remodeling, and alterations in tumor cell migration and invasion capacities, in connection with the accumulation of succinate, the substrate of SDH.

The succinate dehydrogenase inhibitors (SDHi) are fungicides used to control the proliferation of pathogenic fungi in cereal, fruit and vegetable crops, with a mode of action based on blocking the activity of SDH. The analysis of literature data shows that the impact of SDHi on health remains largely unexplored to date, despite a growing number of studies reporting toxic effects in non-target organisms. This is supported by our recent work highlighting 1) the high degree of conservation of the SDH catalytic site (i.e. the SDHi binding site) during the evolution and 2) the ability of SDHi to inhibit SDH in the mitochondria of non-target species, including humans (PMID: 31697708). These observations show that SDHi are not specific to fungal SDH and that their use may present a risk to human health, particularly in the context of chronic exposure through the diet. Moreover, the analysis of regulatory assessment reports shows that most SDHi induce tumors in animals without evidence of genotoxicity. Thus, for these substances, the mechanisms of carcinogenicity are, to date, not clearly established.

Our hypothesis is that, if SDHi fungicides are able to alter SDH activity in humans, the consequences of SDHi exposure on cellular and mitochondrial functions may resemble those observed in SDH-mutated tumors and SDH-deficient cells. We assume that the development of an AOP deciphering the different steps leading to cancer following a genetically-SDH inactivation could help to propose the exploration of relevant key events and adverse effects upon chronic exposure to SDHi fungicides.

Strategy

The development strategy for this AOP is based on the current knowledge on molecular and cellular events triggered by a genetic inactivation of SDH, and on the hypothesis that a chemical SDH inactivation may lead to similar events.

This AOP will be part of the development of an AON with AOP 534 and AOP 474.

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
	MIE	2118	Succinate dehydrogenase, inhibited	SDH, inhibited
	KE	2243	Succinate Accumulation	Succinate Accumulation
	KE	798	Inhibition, Prolyl hydroxylases	Inhibition, Prolyl hydroxylases
	KE	590	N/A, hypoxia	N/A, hypoxia
	AO	885	Increase, Cancer	Increase, Cancer

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Succinate dehydrogenase, inhibited	adjacent	Succinate Accumulation	High	High
Succinate Accumulation	adjacent	Inhibition, Prolyl hydroxylases	High	High
Inhibition, Prolyl hydroxylases	adjacent	N/A, hypoxia	High	High
N/A, hypoxia	adjacent	Increase, Cancer	High	High

Stressors

Name	Evidence
Boscalid	
Bixafen	
Sedaxane	

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Adult	High

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human and other cells in culture	human and other cells in culture	High	NCBI

Sex Applicability

Sex	Evidence
Unspecific	Moderate

References

Appendix 1

List of MIEs in this AOP

Event: 2118: Succinate dehydrogenase, inhibited

Short Name: SDH, inhibited

Event Component

Process	Object	Action
succinate dehydrogenase activity		decreased
FAD metabolic process	succinate dehydrogenase complex	decreased
succinate metabolic process	succinate dehydrogenase complex	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:457 - Succinate dehydrogenase inhibition leading to increased insulin resistance through reduction in circulating thyroxine	MolecularInitiatingEvent
Aop:534 - Succinate dehydrogenase (SDH) inhibition leads to oxidative stress	MolecularInitiatingEvent
Aop:474 - Succinate dehydrogenase inactivation leads to cancer by promoting EMT	MolecularInitiatingEvent
Aop:546 - Succinate dehydrogenase inactivation leads to cancer through hypoxic-like mechanisms	MolecularInitiatingEvent
Aop:588 - Inhibition of the mitochondrial complex II of nigro-striatal neurons leads to parkinsonian motor deficits	MolecularInitiatingEvent

Biological Context

Level of Biological Organization

Molecular

Cell term

Cell term

hepatocyte

Organ term

Organ term
liver parenchyma

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult	High

Sex Applicability

Sex	Evidence
Male	High

SDH inhibition by phthalate esters has been measured and quantified in mitochondria of hepatocytes of adult male CD rats (Melnick and Schiller, 1982; Melnick and Schiller, 1985). Inter-species differences in SDH structure may lead to different susceptibilities in different taxa.

SDH inhibition has been demonstrated by lonidamine, 3-nitroproprionic acid (3-NPA) and 2-thenoyltrifluoroacetone (TTFA) in DB-1, HepG2, HCT116 and HeLa cells, and by lonidamine in mitochondria isolated from adult mouse liver (Guo et al, 2016).

Key Event Description

Eukaryotic succinate dehydrogenase (SDH, EC1.3.5.1 (Brenda, IntEnz)) is an enzyme complex comprising four polypeptide chains (SDHA - SDHD) with associated FAD, Fe-S and haem prosthetic groups that catalyses the reversible oxidation (dehydrogenation) of succinate to fumarate with concomitant reduction of ubiquinone to ubiquinol, serving to channel reducing equivalents from succinate, a tricarboxylic acid (TCA) cycle intermediate, to ubiquinol, an intermediate of the mitochondrial electron transfer chain (Du et al, 2023).

The overall reaction:

succinate + ubiquinone = fumarate + ubiquinol

comprises two, reversible half-reactions:

(1) succinate + FAD = fumarate + $FADH_2$

and:

(2) $FADH_2$ + ubiquinone = FAD + ubiquinol

each of which is catalysed at a different active site.

The active site of reaction 1 is in the hydrophilic protein SDHA that contains the covalently bound FAD group, and protudes from the inner mitochondrial membrane (IMM) into the mitochondrial matrix, making it available to exchange succinate and fumarate within the TCA cycle. The active site of reaction 2 is in a more hydrophobic region comprising transmembrane domains of proteins SDHC and SCHD that insert complex II into the IMM (Du et al, 2023), making it available to ubiquinol and ubiquinone shuttling within the IMM.

The presence of two distinct and different active sites enables SDH inibition to be effected in at least two ways: by inhibition of either active site, with potentially different biochemical and physiological consequences, and by inhibitors with differing characteristics.

Inhibition of SDH can result in reduction of mitochondrial electron transport, and subsequent inhibition of oxidative phosphorylation (e.g. Chen et al, 2021), and also generation of superoxide in the mitochondria, leading to with subsequently deleterious effects such as initiation of apoptosis or necrosis (Murphy et al, 2009).

How it is Measured or Detected

Succinate dehydrogenase activity is generally measured by the spectrophotometric detection of colour change in the presence of an electron acceptor, with succinate (succinic acid) as substrate. Alteration in rate of colour change in the presence of a putative inhibitor determining the strength of that inhibition. The fact that the overall reaction is comprised of two consecutive sub-reactions enables the rate of each sub-reaction - and their inhibition - to be measured separately by appropriate choice of electron acceptor in the presence of succinate as a substrate (e.g. Miyadera et al, 2003). Activities are frequently measured in isolated mitochondria, in order to reduce interference by

extra-cytosolic enaymes and intermediates; mitochondria can be sonicated to obviate rate limitation by mitochondrial upake of succinate (e.g. Guo et al, 2016).

SDH activity

Succinate dehydrogenase (SDH) activity corresponds to reaction (1), above. It can be measured by use of the water-soluble dye 2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-2H-tetrazolium bromide (MTT) in the presence of the intermediate electron carrier phenazine methosulfate (PMS), to intercept electrons before their transport to ubiquinone, and convey them to MTT, which changes colour following its reduction.

SQR activity

Succinate quinone reductase (SQR) activity corresponds to the overall reaction (i.e. 1 and 2), above. It can be measured by reduction of 2,6-dichlorophenolindophenol (DCPIP) in the presence of the 2,3-dimethoxy-6-methyl-1,4-benzoquinone (UQ_2), which accepts electrons from the ubiquinone reduction site and transfers them to DCPIP, thus being a measure of the rate of the entire reaction catalysed by complex II.

References

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Miyadera, H. et al (2003) "Atpenins, potent and specific inhibitors of mitochondrial complex II (succinateubiquinone oxidoreductase)", *Proceedings of the National Academy of Sciences*, Vol 100, pp473-477.

Murphy, M.P. (2009), "How mitochondria produce reactive oxygen species",*Biochemical Journal*, Vol 417, pp1-13.

List of Key Events in the AOP

Event: 2243: Succinate Accumulation

Short Name: Succinate Accumulation

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:474 - Succinate dehydrogenase inactivation leads to cancer by promoting EMT	KeyEvent
Aop:546 - Succinate dehydrogenase inactivation leads to cancer through hypoxic-like mechanisms	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Event: 798: Inhibition, Prolyl hydroxylases

Short Name: Inhibition, Prolyl hydroxylases

Event Component

Process	Object	Action
catalytic activity	Prolyl hydroxylases	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:122 - Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation	MolecularInitiatingEvent
Aop:546 - Succinate dehydrogenase inactivation leads to cancer through hypoxic-like mechanisms	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Event: 590: N/A, hypoxia

Short Name: N/A, hypoxia

Event Component

Process	Object	Action
hypoxia		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:94 - Sodium channel inhibition leading to congenital malformations	KeyEvent
Aop:546 - Succinate dehydrogenase inactivation leads to cancer through hypoxic-like mechanisms	KeyEvent

Biological Context

Level of Biological Organization
Tissue

List of Adverse Outcomes in this AOP

Event: 885: Increase, Cancer

Short Name: Increase, Cancer

Event Component

Process	Object	Action
	Neoplasms	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:141 - Alkylation of DNA leading to cancer 2	AdverseOutcome
Aop:139 - Alkylation of DNA leading to cancer 1	AdverseOutcome
Aop:505 - Reactive Oxygen Species (ROS) formation leads to cancer via inflammation pathway	AdverseOutcome
Aop:513 - Reactive Oxygen (ROS) formation leads to cancer via Peroxisome proliferation-activated receptor (PPAR) pathway	AdverseOutcome
Aop:474 - Succinate dehydrogenase inactivation leads to cancer by promoting EMT	AdverseOutcome
Aop:546 - Succinate dehydrogenase inactivation leads to cancer through hypoxic-like mechanisms	AdverseOutcome

Biological Context

Level of Biological Organization

Tissue

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
Mus musculus	Mus musculus	High	NCBI
Rattus norvegicus	Rattus norvegicus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Life Stage: All life stages. Older individuals are more likely to manifest this key event (adults > juveniles > embryos).

Sex: Applies to both males and females.

Taxonomic: Appears to be present broadly, with representative studies including mammals (humans, lab mice, lab rats), teleost fish, and invertebrates (cladocerans, mussels).

Key Event Description

Cancer is a general key event for related diseases each exhibiting uncontrolled proliferation of abnormal cells (for review see Hanahan and Weinberg 2011). A cancer often is initially associated with a specific organ, with malignant tumors developing ability to metastasize, or travel to other areas of the body. Most cancers develop from genetic mutations in normal cells, although a minority of cancers are hereditary. Exposure to chemical stressors, radiation, tobacco smoke, or viruses can increase the likelihood that cancer will develop.

Cancer cells proliferate due to capabilities summarized by Hanahan and Weinberg (2011):

1. Sustained proliferation signaling – by deregulating normal cell signals, cancer cells can sustain chronic proliferation.
2. Evading growth suppressors – by evading activities of tumor suppressor genes, cancer cells continue to proliferate.
3. Activating invasion and metastasis – by altering shape and attachment to cells in the extracellular matrix, cancer cells gain ability to move to other locations.
4. Enabling replicative immortality – by disabling senescence pathways, cancer cells have extended lifespans.
5. Inducing angiogenesis – by enabling neovasculature, cancer cells receive nutrients and oxygen and get rid of waste products.
6. Resisting cell death – by evading apotosis and necrosis defense pathways, cancer cells avoid elimination.

How it is Measured or Detected

Most carcinogenicity studies are conducted with rodents (see OECD 2018; Zhou et al. 2023 for methods) or in-vitro with mammalian cell lines (see OECD 2023 for methods). Cancer is usually detected by biopsy or histopathological examination of tissue. Gene expression levels can also be assessed, as increased transcription of known genes have been associated with specific cancers (ex. Tumor Necrosis Factor (Pavet et al. 2014); Heat Shock Factors (Vihervaara and Sistonen 2014; Androgen Receptor (Heinlein and Chang 2004)).

Regulatory Significance of the AO

Cancer is a critical endpoint in human health risk assessment. It is embedded in regulatory frameworks for human health protection in many countries (see OSHA 2023 for examples of US regulations and European Parliament 2022 for examples of regulations in Europe).

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Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 3302: SDH, inhibited leads to Succinate Accumulation

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Succinate dehydrogenase inactivation leads to cancer by promoting EMT	adjacent	High	High
Succinate dehydrogenase inactivation leads to cancer through hypoxic-like mechanisms	adjacent	High	High

Relationship: 3304: Succinate Accumulation leads to Inhibition, Prolyl hydroxylases

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Succinate dehydrogenase inactivation leads to cancer through hypoxic-like mechanisms	adjacent	High	High

Relationship: 3369: Inhibition, Prolyl hydroxylases leads to N/A, hypoxia

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Succinate dehydrogenase inactivation leads to cancer through hypoxic-like mechanisms	adjacent	High	High

Relationship: 3370: N/A, hypoxia leads to Increase, Cancer

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Succinate dehydrogenase inactivation leads to cancer through hypoxic-like mechanisms	adjacent	High	High