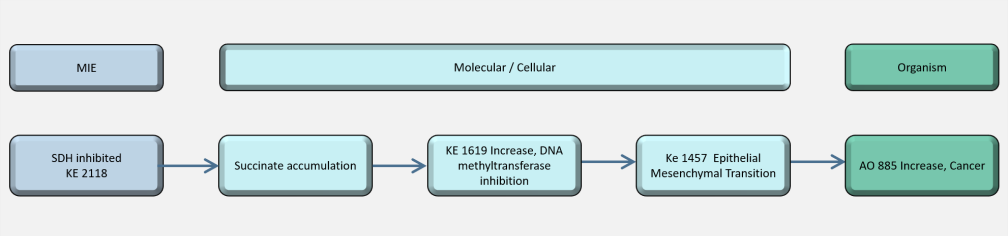
AOP ID and Title:

AOP 474: Succinate dehydrogenase inactivation leads to cancer by promoting EMT
Short Title: SDH inactivation, DNA methyltransferase inhibition, EMT and cancer

Graphical Representation



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Status

Author status	OECD status	OECD project	SAAOP status
Under development: Not open for comment. Do not cite	Under Development	1.106	Included in OECD Work Plan

Coaches

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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 (PMID: 37778286).

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 (PMID: 33112834)

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 (PMID: 40285898).

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 (PMID: 37778286). 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.

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	1619	Increase, DNA methyltransferase inhibition	Increase, DNMT inhibition
	KE	1457	Epithelial Mesenchymal Transition	EMT
	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	Increase, DNA methyltransferase inhibition	High	High
Increase, DNA methyltransferase inhibition	adjacent	Epithelial Mesenchymal Transition	Moderate	Moderate
Epithelial Mesenchymal Transition	adjacent	Increase, Cancer	High	High

Stressors

Name	Evidence
Boscalid	
Bixafen	

Name	Evidence
Sedaxane	

Overall Assessment of the AOP

This AOP describes the molecular and cellular consequences of succinate dehydrogenase (SDH) inactivation, leading to adverse biological outcomes specifically cancer. It integrates evidence from genetic and chemical inhibition models, supporting the hypothesis that chemical inactivation of SDH can mimic the effects observed in genetic SDH deficiencies.

This AOP is broadly applicable across multiple taxa, as SDH is a highly conserved enzyme among eukaryotes. The key events have been observed in mammalian models (rodents, humans) as well as in some invertebrate systems, though most empirical evidence comes from vertebrate studies. The AOP is relevant to both sexes, as SDH-related dysfunctions have not shown sex-specific susceptibility.

Regarding life stage, available data suggest that embryonic and juvenile stages may be more vulnerable to SDH inactivation due to their higher metabolic demands and reliance on mitochondrial function. However, further studies are needed to delineate life-stage-dependent sensitivities.

The **overall weight of evidence** for this AOP is moderate to strong, with well-established mechanistic understanding of early molecular events but some uncertainties in later-stage key event relationships.
- Biological plausibility: Strong, as SDH inactivation is well-documented to disrupt mitochondrial metabolism, leading to accumulation of oncometabolites (e.g., succinate) and altered cellular signaling. SDH inactivation is clearly associated with the occurence of multiple cancers.
- Empirical support: Moderate to strong, particularly for the initial key events. In vitro and in vivo studies demonstrate consistency in key event progression, but quantitative understanding of dose-response relationships remains limited.
- Concordance and consistency: Strong, with observed reproducibility across multiple experimental models, despite moderate species-specific differences.

Regulatory Considerations
This AOP contributes to a broader Adverse Outcome Network (AON) alongside AOP 534, providing a mechanistic basis for regulatory applications such as:
- Priority setting: identifying chemicals that may interfere with SDH function as potential environmental and occupational hazards. This is the case for SDHi fungicides.
- Testing strategies: informing the development of *in vitro* assays targeting early key events to reduce reliance on animal models (SDH activity)
- Risk assessment: Supporting the integration of mitochondrial toxicity (mitotoxicity) into chemical risk assessment frameworks, particularly for compounds suspected of disrupting cellular metabolism.

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

This AOP is applicable across multiple biological domains, considering its relevance to various taxa, sexes, life stages, and broader biological contexts. The key molecular and cellular events triggered by SDH inactivation are largely conserved across eukaryotes, making this AOP broadly applicable.

Sex Applicability
This AOP is considered applicable to both sexes, as no significant sex-specific differences have been reported in SDH inactivation studies. While hormonal and metabolic differences could theoretically modulate susceptibility in certain contexts, available data do not indicate a strong sex-dependent effect. Further research may be needed to assess potential sex-specific variations in sensitivity.

Life Stage Applicability
The consequences of SDH inactivation are relevant across multiple life stages, but susceptibility may vary.
- Early developmental stages (embryonic, juvenile): more vulnerable due to higher metabolic demands and reliance on mitochondrial function for growth and differentiation. Disruptions in energy metabolism during early development

may lead to severe consequences, including impaired organogenesis.

- Adult stage: the impact of SDH inhibition is likely tissue-specific, with high-energy-demand organs (e.g., brain, heart, muscle) being particularly affected. While metabolic plasticity in adults may provide some resilience, chronic SDH inhibition can still lead to progressive dysfunction, particularly in tissues with limited regenerative capacity.

- Vulnerable stage: furthermore, the invalidation or inhibition of SDH in already vulnerable populations is likely to aggravate their pathophysiological condition. Furthermore, the invalidation or inhibition of SDH in already vulnerable populations is likely to aggravate their pathophysiological condition.

Taxonomic Applicability
SDH is an essential enzyme in the mitochondrial electron transport chain, highly conserved across eukaryotic species. Strong empirical evidence supporting this AOP comes from mammalian models (rodents, humans), where SDH inactivation has been linked to metabolic dysregulation and disease. Additionally, SDH impairment has been studied in invertebrate models (Drosophila melanogaster, Caenorhabditis elegans), demonstrating similar disruptions in mitochondrial function and cellular signaling (https://doi.org/10.1016/j.jmb.2009.02.028 ; PMID: 24954416). While fundamental mechanisms are preserved across species, variations in metabolic compensation and stress response pathways may influence the severity of downstream effects in different taxa.

Other Biological Contexts
- Cell and tissue specificity: the effects of SDH inactivation are particularly relevant in metabolically active tissues such as the nervous system, cardiac and skeletal muscles, and endocrine organs, where mitochondrial function is critical for normal physiological processes.

- Physiological and pathological conditions: this AOP is especially relevant in the context of mitochondrial diseases, neurodegenerative disorders, and cancer, where SDH dysfunction has been implicated. The findings may also have implications for toxicological assessments of environmental and pharmaceutical compounds targeting mitochondrial function.

Essentiality of the Key Events

The essentiality of each Key Event (KE) was assessed based on experimental evidence demonstrating whether its manipulation (e.g., inhibition, knockdown, overexpression, or supplementation) prevents the occurrence of subsequent KEs and/or the final Adverse Outcome (AO).

In this AOP, the Molecular Initiating Event (MIE) is the inhibition of succinate dehydrogenase (SDH, KE 2118), which leads to succinate accumulation (KE2243), inhibition of DNA methyltransferase (DNMT) activity (KE 1619), and activation of epithelial–mesenchymal transition (EMT, KE 1457). Strong evidence supports the essentiality of SDH inhibition and EMT activation, while succinate accumulation and DNMT inhibition are also supported by experimental data, although the direct causal links have varying strength depending on the studies. Overall, the evidence suggests that preventing any of these KEs can significantly alter the progression toward the AO.

Key Event (KE)	Essentiality Level	Evidence Summary	References
KE 2118 - SDH inhibited	**Strong**	The SDH complex uses succinate as a substrate. Invalidation, genetic knockdown or inhibition of SDH leads to succinate accumulation. Restoration of SDH activity normalizes mitochondrial function and blocks epigenetic and phenotypic changes, leading to EMT.	PMID: 40359599; PMID: 33112834
KE 2243 - Succinate accumulation	**Moderate**	Exogenous succinate administration can induce epigenetic alterations and EMT markers in some in vitro models, but effects are partial and context-dependent. Direct suppression of succinate accumulation to block downstream KEs is less frequently reported.	REF
KE 1619 - Increase, DNA methyltransferase inhibition	**Strong**	Elevated succinate inhibits α-KG-dependent dioxygenases (including TET enzymes), resulting in altered DNA methylation patterns. DNMT inhibition or knockdown experimentally reproduces these patterns and related gene expression changes. Conversely, DNMT overexpression or α-KG supplementation can attenuate these epigenetic effects.	REF
KE 1457 - Epithelial Mesenchymal Transition (EMT)	**Strong**	Pharmacological or genetic inhibition of EMT-associated transcription factors (e.g., SNAI1, TWIST) blocks tumor progression and cell migration, even in the presence of upstream perturbations such as SDH inhibition or succinate accumulation.	REF

Weight of Evidence Summary

KER1: SDH inhibited (KE 2118) → Succinate accumulation (KE 2243)
Strong. SDH catalyzes oxidation of succinate to fumarate in the TCA cycle; loss or inhibition of SDH predictably causes intracellular succinate build-up. This is well supported in SDH-deficient tumors and models (REF)

KER2: Succinate accumulation (KE 2243) → Increase, DNA methyltransferase (DNMT) inhibition (KE 1619)

Mixed. Robust evidence shows succinate competitively inhibits α-KG–dependent dioxygenases (e.g., TET DNA demethylases, Jumonji histone demethylases), leading to DNA hypermethylation in SDH-deficient tumors. However, this mechanism reflects TET inhibition, not DNMT inhibition. Direct evidence that succinate inhibits DNMT enzymes is limited and, mechanistically, DNMT inhibition would be expected to favor hypomethylation, opposite to what is seen in SDH-deficient contexts. Thus, the biological plausibility is strong for "succinate → inhibition of α-KG–dependent demethylases (e.g., TET) → hypermethylation," but weak/inconsistent for "succinate → DNMT inhibition."

KER3: Increase, DNMT inhibition (KE 1619) → Epithelial–Mesenchymal Transition (EMT, KE 1457)
Moderate/conditional. EMT programs are influenced by epigenetic remodeling (e.g., methylation at CDH1 and other epithelial genes). There is ample evidence that increased DNA methylation promotes EMT and invasion, and that epigenetic drugs can modulate EMT. But again, most mechanistic support involves hyper-methylation via TET inhibition or DNMT activity supporting EMT—whereas "DNMT inhibition" would usually counter hypermethylation. Therefore, the general epigenetic control → EMT is biologically plausible, but the directionality specified by KE 1619 ("DNMT inhibition") is poorly aligned with the SDH/succinate epigenetic signature.

(Non-adjacent reinforcement): Independent lines link SDH loss/succinate accumulation to EMT-like phenotypes via pseudohypoxia (PHD/HIF), ROS, and epigenetic routes; exogenous succinate can enhance EMT-related functions in several models.

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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Du, Z. et al (2023) "Structure of the human respiratory complex II",*Proceedings of the National Academy of Sciences*", Vol 120, e2216713120.

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Melnick, R.L. and Schiller, C.M. (1985), "Effect of phthalate esters on energy coupling and succinate oxidation in rat liver mitochondria", *Toxicology*, Vol 34, pp13-27.

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: 1619: Increase, DNA methyltransferase inhibition

Short Name: Increase, DNMT inhibition

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:336 - DNA methyltransferase inhibition leading to population decline (1)	MolecularInitiatingEvent
Aop:337 - DNA methyltransferase inhibition leading to population decline (2)	MolecularInitiatingEvent
Aop:338 - DNA methyltransferase inhibition leading to population decline (3)	MolecularInitiatingEvent
Aop:339 - DNA methyltransferase inhibition leading to population decline (4)	MolecularInitiatingEvent
Aop:340 - DNA methyltransferase inhibition leading to transgenerational effects (1)	MolecularInitiatingEvent
Aop:341 - DNA methyltransferase inhibition leading to transgenerational effects (2)	MolecularInitiatingEvent
Aop:474 - Succinate dehydrogenase inactivation leads to cancer by promoting EMT	KeyEvent

Biological Context

Level of Biological Organization

Molecular

Event: 1457: Epithelial Mesenchymal Transition

Short Name: EMT

Event Component

Process	Object	Action
epithelial to mesenchymal transition	Epithelial cell	occurrence

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:241 - Latent Transforming Growth Factor beta1 activation leads to pulmonary fibrosis	KeyEvent
Aop:206 - Peroxisome proliferator-activated receptors γ inactivation leading to lung fibrosis	KeyEvent
Aop:280 - α-diketone-induced bronchiolitis obliterans	KeyEvent
Aop:347 - Toll-like receptor 4 activation and peroxisome proliferator-activated receptor gamma inactivation leading to pulmonary fibrosis	KeyEvent
Aop:414 - Aryl hydrocarbon receptor activation leading to lung fibrosis through TGF-β dependent fibrosis toxicity pathway	KeyEvent
Aop:415 - Aryl hydrocarbon receptor activation leading to lung fibrosis through IL-6 toxicity pathway	KeyEvent
Aop:443 - DNA damage and mutations leading to Metastatic Breast Cancer	KeyEvent
Aop:298 - Increase in reactive oxygen species (ROS) leading to human treatment-resistant gastric cancer	KeyEvent
Aop:452 - Adverse outcome pathway of PM-induced respiratory toxicity	KeyEvent
Aop:474 - Succinate dehydrogenase inactivation leads to cancer by promoting EMT	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Cell term

Cell term
epithelial cell

Organ term

Organ term
organ

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
humans	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Not Otherwise Specified	Not Specified

Sex Applicability

Sex	Evidence
Unspecific	Not Specified

The key event is applicaple in *Homo sapiens*:

- Wnt5a expression leads to epithelial-mesenchymal transition (EMT) and metastasis in non-small-cell lung cancer in *Homo sapiens* (Wang et al., 2017).
- WNT2 expression lead to EMT induction in *Homo sapiens* (Zhou et al., 2016).
- EMT is induced in cancer and involved in cancer metastasis in*Homo sapiens* (Suarez-Carmona, Lesage, Cataldo, & Gilles, 2017) (Du & Shim, 2016).

Regulation of miRNA expression by DNA replication,damage and repair responses,transcription and translation has been proved in animals like mice,canine and cell line experiments.

Key Event Description

Epithelial-mesenchymal transition (EMT) is a phenomenon in which the cells transit from epithelial-like into mesenchymal-like phenotypes (Huan et al., 2022; Tanabe, 2017; Tanabe et al., 2015). In cancer, cells exhibiting EMT features contribute to metastasis and drug resistance.

It is known that D-2-hydroxyglurate induces EMT (Guerra et al., 2017; Jia et al., 2018; Mishra et al., 2018; Sciacovelli & Frezza, 2017). D-2-hydroxyglurate, an inhibitor of Jumonji-family histone demethylase, increased the trimethylation of histone H3 lysine 4 (H3K4) in the promoter region of the zinc finger E-box-binding homeobox 1 (ZEB1), followed by the induction of EMT (Colvin et al., 2016).

Wnt5a induces EMT and metastasis in non-small-cell lung cancer (Wang et al., 2017).

EMT is related to Wnt/beta-catenin signaling and is important for treatment-resistant cancer (Tanabe et al., 2016).

TGFbeta induces EMT (Wendt et al., 2010).

ZEB is one of the critical transcription factors for EMT regulation (Zhang et al., 2015).

SNAI1 (Snail) is an important transcription factor for cell differentiation and survival. The phosphorylation and nuclear localization of Snail1 induced by Wnt signaling pathways are critical for the regulation of EMT (Kaufhold & Bonavida, 2014).

Transcription factors SNAI1 and TWIST1 induce EMT (Hodge et al., 2018) (Mani et al., 2008).

It is suggested that Sp1, a transcription factor involved in cell growth and metastasis, is induced by cytochrome P450 1B1 (CYP1B1), and promotes EMT, which leads to cell proliferation and metastasis (Kwon et al., 2016).

Biological state

An epithelial-mesenchymal transition (EMT) is a biologic process in which epithelial cells are polarized, interact through their basal surface with basement membrane, and undergo biochemical changes to assume a mesenchymal cell phenotype.

This phenotypic transformation has various characters such as enhanced migratory capacity, high invasiveness, elevated resistance to apoptosis, and greatly increased production of ECM components (Kalluri, R., and Neilson, E.G. 2003). The completion of an EMT is signalled by the degradation of the underlying basement membrane and the formation of a mesenchymal cell that can migrate away from the epithelial layer in which it originated.

EMT has a number of distinct molecular processes like activation of transcription factors, expression of specific cell surface proteins, reorganization and expression of cytoskeletal proteins, production of ECM-degrading enzymes, and changes in the expression of specific microRNAs. These factors are used as biomarkers to demonstrate the passage of a cell through an EMT.

Biological compartment

Cellular

Role in General Biology:

Excessive proliferation of epithelial cells and angiogenesis mark the initiation and early growth of primary epithelial cancers. (Hanahan, D., and Weinberg, R.A. 2000). The subsequent acquisition of invasiveness, initially manifest by invasion through the basement membrane, is thought to herald the onset of the last stages of the multi-step process that leads eventually to metastatic dissemination, with life-threatening consequences. There has been an intense research going on in the genetic controls and biochemical mechanisms underlying the acquisition of the invasive phenotype and the subsequent systemic spread of the cancer cell. Activation of an EMT program has been proposed as the critical mechanism for the acquisition of malignant phenotypes by epithelial cancer cells (Thiery, J.P. 2002).

Pre-clinical experiments such as mice models and cell culture experiments has demonstrated that carcinoma cells can acquire a mesenchymal phenotype and express mesenchymal markers such as α-SMA, FSP1, vimentin, and desmin (Yang, J., and Weinberg, R.A. 2008). These cells are seen at the invasive front of primary tumors and are considered to be the cells that eventually enter into subsequent steps of the invasion-metastasis cascade, i.e., intravasation, transport through the circulation, extravasation, formation of micro metastases, and ultimately colonization (the growth of small colonies into macroscopic metastases) (Thiery, J.P. 2002, Fidler, I.J., and Poste, G. 2008, Brabletz, T., et al. 2001).

An apparent paradox comes from the observation that the EMT-derived migratory cancer cells typically establish secondary colonies at distant sites that resemble, at the histopathological level, the primary tumor from which they arose; accordingly, they no longer exhibit the mesenchymal phenotypes ascribed to metastasizing carcinoma cells. Reconciling this behaviour with the proposed role of EMT as a facilitator of metastatic dissemination requires the additional notion that metastasizing cancer cells must shed their mesenchymal phenotype via a MET during the course of secondary tumor formation (Zeisberg, M et al 2005). The tendency of disseminated cancer cells to undergo EMT likely reflects the local microenvironments that they encounter after extravasation into the parenchyma of a distant organ, quite possibly the absence of the heterotypic signals they experienced in the primary tumor that were responsible for inducing the EMT in the first place (Thiery, J.P. 2002, Jechlinger, M et al 2002, Bissell, M.J et al 2002). These evidences indicate that induction of an EMT is likely to be a centrally important mechanism for the progression of carcinomas to a metastatic stage and implicates MET during the subsequent colonization process. However,

many steps of this mechanistic model still require direct experimental validation. It remains unclear at present whether these phenomena and molecular mechanisms relate to and explain the metastatic dissemination of non-epithelial cancer cells.

The entire spectrum of signaling agents that contribute to EMTs of carcinoma cells remains unclear. One theory suggests that the genetic and epigenetic alterations undergone by cancer cells during the course of primary tumor formation render them especially responsive to EMT-inducing heterotypic signals originating in the tumor-associated stroma. Oncogenes induce senescence, and recent studies suggest that cancer cell EMTs may also play a role in preventing senescence induced by oncogenes, thereby facilitating subsequent aggressive dissemination (Smit, M.A., and Peeper, D.S. 2008, Ansieau, S., et al. 2008, Weinberg, R.A. 2008). In the case of many carcinomas, EMT-inducing signals emanating from the tumor-associated stroma, notably HGF, EGF, PDGF, and TGF-β, appear to be responsible for the induction or functional activation in cancer cells of a series of EMT-inducing transcription factors, notably Snail, Slug, zinc finger E-box binding homeobox 1 (ZEB1), Twist, Goosecoid, and FOXC2 (Thiery, J.P. 2002, Jechlinger, M et al 2002, Shi, Y., and Massague, J. 2003, Niessen, K., et al. 2008, Medici, D et al 2008, Kokudo, T., et al. 2008). Once expressed and activated, each of these transcription factors can act pleiotropically to choreograph the complex EMT program, more often than not with the help of other members of this cohort of transcription factors. The actual implementation by these cells of their EMT program depends on a series of intracellular signaling networks involving, among other signal- transducing proteins, ERK, MAPK, PI3K, Akt, Smads, RhoB, β-catenin, lymphoid enhancer binding factor (LEF), Ras, and c-Fos as well as cell surface proteins such as β4 integrins, α5β1 integrin, and αVβ6 integrin (Tse, J.C., and Kalluri, R. 2007). Activation of EMT programs is also facilitated by the disruption of cell-cell adherens junctions and the cell-ECM adhesions mediated by integrins (Yang, J., and Weinberg, R.A. 2008, Weinberg, R.A. 2008, Gupta, P.B et al 2005, Yang, J et al 2006, Mani, S.A., et al. 2007, Mani, S.A., et al. 2008, Hartwell, K.A., et al. 2006, Taki, M et al 2006)..

How it is Measured or Detected

Loss of E-cadherin and cell polarity is considered to be a fundamental event in epithelial-mesenchymal transition. The simultaneous expression of epithelial (e.g. E-cadherin) and mesenchymal markers (e.g. N-cadherin and vimentin) within the airway epithelium are indicative for ongoing transition (Borthwick et al. 2009, 2010).

	Method/ measurement referenc	Reliability	Strength of evidence	Assay fit for purpose	Repeatability/ reproducibility	Direct measure
Human cell line	qRT-PCR,cell viability assay, Western blotting,EdU incorporation assay	+	Strong	Yes	Yes	Yes
Human	IHC,micro array,qPCR, SNP array	+	Moderate	Yes	Yes	Yes

- EMT can be detected by immunostaining with pro-surfactant protein-C (pro-SPC) and N-cadherin in idiopathic pulmonary fibrosis (IPF) lung *in vivo* (Kim et al., 2006).
- EMT can be detected by immunostaining with vimentin in lung alveola*in vivo* (Kim et al., 2006).
- EMT can be detected as the increased level of the transcription factors, zinc finger E-box-binding homeobox (ZEB), Twist and Snail (Huang et al., 2022).

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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: 3303: Succinate Accumulation leads to Increase, DNMT inhibition

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Succinate dehydrogenase inactivation leads to cancer by promoting EMT	adjacent	High	High

Relationship: 3305: Increase, DNMT inhibition leads to EMT

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Succinate dehydrogenase inactivation leads to cancer by promoting EMT	adjacent	Moderate	Moderate

Relationship: 3306: EMT leads to Increase, Cancer

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Succinate dehydrogenase inactivation leads to cancer by promoting EMT	adjacent	High	High