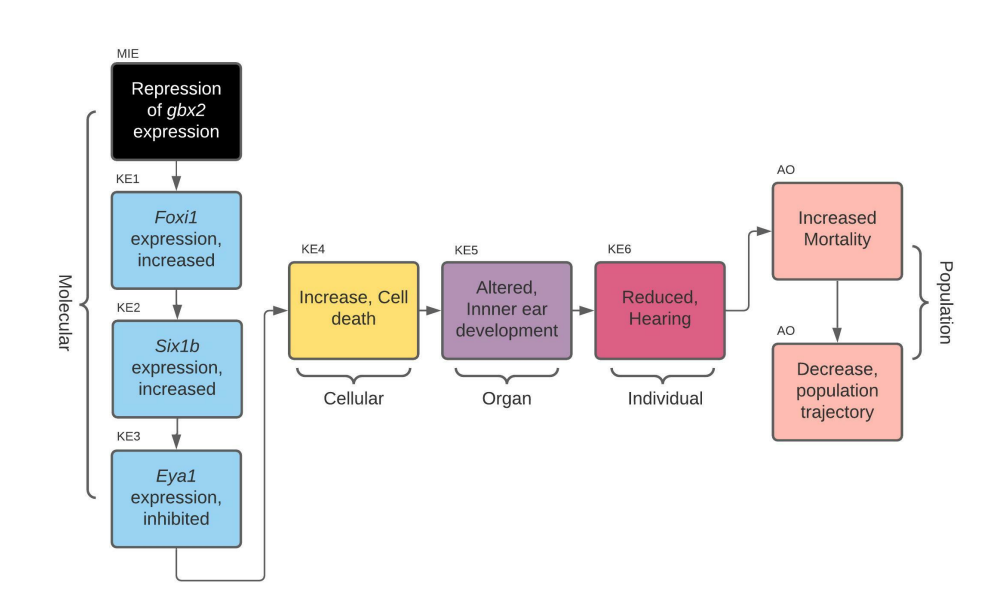
AOP ID and Title:

AOP 410: Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality
Short Title: Repression of Gbx2 expression leads to increased mortality

Graphical Representation



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Status

Author status	OECD status	OECD project	SAAOP status
Under development: Not open for comment. Do not cite			

Background

The motivation behind building the AOP was methodological. Our team has recently developed molecular causal networks for developmental cardiotoxicity and neurotoxicity in zebrafish (doi.org/10.1021/acs.chemrestox.0c00095). These networks are highly curated, but rather large, going from adverse outcomes on the organ level upstream to wherever evidence takes us (many times finishing at what would be called MIEs). As there are many causal networks already present on the http://causalbionet.com/ (mostly for humans and for lung conditions), we were wondering how the rich knowledge available in causal pathways could be translated to AOPs. The AOP described in this document is one such example.

Summary of the AOP

Events

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

Sequence	Type	Event ID	Title	Short name
	MIE	1647	GSK3beta inactivation	GSK3beta inactivation
1	MIE	1902	Repression of Gbx2 expression	Repression of Gbx2 expression
2	KE	1903	foxi1 expression, increased	foxi1 expression, increased
3	KE	1904	six1b expression, increased	six1b expression, increased
4	KE	1905	eya1 expression, inhibited	eya1 expression, inhibited

Sequence	Type	Event ID	Title	Short name
5	KE	1825	Increase, Cell death	Increase, Cell death
6	KE	1930	altered, inner ear development	Altered, inner ear development
7	KE	1008	Reduced, Hearing	Reduced, Hearing
8	KE	351	Increased Mortality	Increased Mortality

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
GSK3beta inactivation	adjacent	Repression of Gbx2 expression	High	Low
Repression of Gbx2 expression	adjacent	foxi1 expression, increased	Moderate	Not Specified
foxi1 expression, increased	adjacent	six1b expression, increased	Moderate	Not Specified
six1b expression, increased	adjacent	eya1 expression, inhibited	Moderate	Not Specified
eya1 expression, inhibited	adjacent	Increase, Cell death	Moderate	Not Specified
Increase, Cell death	adjacent	altered, inner ear development	Moderate	Low
altered, inner ear development	adjacent	Reduced, Hearing	High	Low
Reduced, Hearing	adjacent	Increased Mortality	High	High

Overall Assessment of the AOP

References

Appendix 1

List of MIEs in this AOP

Event: 1647: GSK3beta inactivation

Short Name: GSK3beta inactivation

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:410 - Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	MolecularInitiatingEvent

Stressors

Name
CHIR99021
BIO (6-bromoindirubin-3'-oxime)
Kenpaullone
SB216763
TWS119
CHIR98014

Biological Context

Level of Biological Organization

Molecular

Cell term

Cell term

cell

Organ term

Organ term

organ

Evidence for Perturbation by Stressor

CHIR99021

CHIR99021 inhibits GSK3beta (Wu et al., 2015) .

BIO (6-bromoindirubin-3’-oxime)

BIO (6-bromoindirubin-3’-oxime) inhibits GSK3beta (Wu et al., 2015).

Kenpaullone

Kenpaullone inhibits GSK3beta (Yang et al., 2013).

SB216763

SB216763 inhibits GSK3betat (Naujok, Lentes, Diekmann, Davenport, & Lenzen, 2014).

TWS119

TWS119 inhibits GSK3beta (Tang et al., 2018).

CHIR98014

CHIR98014 inhibits GSK3beta (Guerrero et al., 2014; Lian et al., 2014).

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
zebra fish	Danio rerio	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Phosphorylation of GSK3beta is induced, which means the inactivation of GSK3beta, in *Homo sapiens* (Huang et al., 2019). Evidence for this KE is also provided for zebrafish (Anichtchik et al., 2008; Wang et al. 2018)

Key Event Description

The protein encoded by *gsk3b* gene is a serine-threonine kinase belonging to the glycogen synthase kinase subfamily. It is a negative regulator of glucose homeostasis and is involved in energy metabolism, inflammation, ER-stress, mitochondrial dysfunction, and apoptotic pathways.

Defects in this gene have been associated with Parkinson disease and Alzheimer disease (*GSK3B Gene - GeneCards*). GSK3b has been identified within mitochondria (Hoshi et al., 1996), as well as in the cytoplasm (Anichtchik *et al.*, 2008).

GSK3b kinase is constitutively active in resting cells and undergoes a rapid and transient inhibition in response to a number of external signals. GSK3b activity is regulated by site-specific phosphorylation. Full activity of GSK3b generally requires phosphorylation at tyrosine 216 (Tyr216), and conversely, phosphorylation at serine 9 (Ser9) inhibits GSK3b activity. Phosphorylation of Ser9 is the most common and important regulatory mechanism. Many kinases are capable of phosphorylating Ser9, including p70 S6 kinase, extracellular signal-regulated kinases (ERKs), p90Rsk (also called MAP-KAP kinase-1), protein kinase B (also called Akt), certainisoforms of protein kinase C (PKC) and cyclic AMP-dependent protein kinase (protein kinase A, PKA). In opposition to the inhibitory modulation of GSK3b that occurs by serine phosphorylation, tyrosine phosphorylation of GSK3b increases the enzyme's activity (Grimes and Jope, 2001; Luo, 2012).

Glycogen synthase kinase 3beta (GSK3 beta) is inhibited by CHIR99021 (C. H. Li et al., 2017; C. C. Liu et al., 2016; Sineva & Pospelov, 2010).

Glycogen synthase kinase 3beta (GSK3 beta) is inhibited by BIO (6-bromoindirubin-3'-oxime) (Mohammed et al., 2016; Sineva & Pospelov, 2010).

Kenpaullone is a dual inhibitor for GSK3 alpha/beta and HPK1/GCK-like kinase (Y. M. Yang et al., 2013; Yao et al., 1999).

CHIR and BIO treatments lead to a slight upregulation of the primary transcripts of the miR-302-367 cluster and miR-181 family of miRNAs, which activate Wnt/beta-catenin signaling (Y. Wu et al., 2015).

SB216763 inhibits GSK3beta (Naujok et al., 2014).

TWS119 inhibits GSK3beta (Tang et al., 2018).

CHIR98014 inhibits GSK3beta (Guerrero et al., 2014; Lian et al., 2014).

How it is Measured or Detected

Inactivation of GSK3 beta is measured by Wnt/beta-catenin activity assay, in which the vector containing the firefly luciferase gene controlled by TCF/LEF binding sites is transfected in the cells (Naujok et al., 2014). Phosphorylation of GSK3beta at residue Ser9 leads to the inactivation of GSK3beta. Phosphorylation of GSK3 beta is measured by immunoblotting with anti-phospho-GSK3beta (Huang et al., 2019).

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Event: 1902: Repression of Gbx2 expression

Short Name: Repression of Gbx2 expression

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:410 - Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	MolecularInitiatingEvent

Stressors

Name
BIO (6-bromoindirubin-3'-oxime)
Retinoic acid
su5402

Biological Context

Level of Biological Organization
Molecular

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

- Zebrafish embryos were treated with chemical inhibitors or activators of various signaling pathways, such as the Wnt, FGF, retinoic acid (RA), HH, BMP, Nodal, and Notch pathways, and examined gbx2 expression in the telencephalon. First, embryos were treated with chemicals from 14 hpf to 18 hpf, immediately before the advent of gbx2 expression in the telencephalon, and then gbx2 expression was examined in this brain region . In embryos treated with BIO, a selective GSK3 inhibitor that activates Wnt signaling (Sato et al., 2004), gbx2 expression was specifically repressed in the telencephalon, but was unaffected or weakly activated in the isthmus and otic vesicle (OV). In embryos where FGF signaling was inhibited by SU5402, gbx2 was downregulated in the telencephalon and MHB, but its expression in the OV was little affected. Retinoic acid (RA) treatment strongly repressed gbx2 expression in the telencephalon, but not in the MHB and OV. These results suggest that gbx2-dependent telencephalon development is regulated by Wnt, FGF, and RA signaling (Z. Wang et al., 2018).
- To clarify the critical stages of previous study for gbx2 regulation in the telencephalon, chemical treatment started between 14 and 17 hpf and gbx2 expression was examined at 18 hpf. Alternatively, chemical treatment was started at 14 hpf and then embryos were washed between 15 and 18 hpf, cultured in the absence of chemicals, and gbx2 expression was examined at 18 hpf. Resuoults showed that the downregulation of gbx2 by BIO grew less significant as the start time was delayed, and the repression of gbx2 by BIO in the telencephalon became less prominent when the chemicals were removed earlier, suggesting that Wnt signaling remains effective throughout the 4-h period (14–18 hpf) and that the repressive effect of BIO is reversible. Similarly, SU5402 mediated repression of gbx2 expression in the telencephalon and MHB became less significant as the treatment start time was delayed from 14 hpf to 17 hpf, and gbx2 expression was gradually restored with earlier removal of the chemical, showing that FGF signaling is continuously required for gbx2 expression in the telencephalon. Essentially the same results were obtained with RA treatment in terms of gbx2 expression in the telencephalon (Z. Wang et al., 2018).

BIO (6-bromoindirubin-3’-oxime)

Embryos were treated with chemicals from 14 hpf to 18 hpf, immediately before the advent of gbx2 expression in the telencephalon, and then gbx2 expression was examined in this brain region . In embryos treated with BIO, a selective GSK3 inhibitor that activates Wnt signaling (Sato et al., 2004), gbx2 expression was specifically repressed in the telencephalon, but was unaffected or weakly activated in the isthmus and otic vesicle (OV).

Retinoic acid

Zebrafish embryos were treated with chemicals from 14 hpf to 18 hpf, immediately before the advent of gbx2 expression in the telencephalon, and then gbx2 expression was examined in this brain region. Retinoic acid (RA) treatment strongly repressed gbx2 expression in the telencephalon, but not in the MHB and OV.

su5402

Zebrafish embryos were treated with chemicals from 14 hpf to 18 hpf, immediately before the advent of gbx2 expression in the telencephalon, and then gbx2 expression was examined in this brain region. In embryos where FGF signaling was inhibited by SU5402, gbx2 was downregulated in the telencephalon and MHB, but its expression in the OV was little affected (Z. Wang et al., 2018).

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Embryo	High

Sex Applicability

Sex	Evidence
Unspecific	High

The gastrulation brain homebox (Gbx) group of transcription factor genes, composed of two genes, gbx1 and gbx2, in vertebrates, is also present in invertebrates (Chiang et al., 1995), and can be regarded as widely conserved among animals (Wang et al., 2018). Gbx2 functions in a variety of developmental processes after midbrain-hindbrain boundary (MHB) establishment. (Burroughs-Garcia et al., 2011) data demonstrate that the role of gbx2 in anterior hindbrain development is functionally conserved between zebrafish and mice. This gene was shown to be required for neural crest (NC) formation in mice (B. Li et al., 2009; Roeseler et al., 2012). In Xenopus gbx2 is the earliest factor for specifying neural crest (NC) cells, and that gbx2 is directly regulated by NC inducing signaling pathways, such as Wnt/β-catenin signaling (Li et al., 2009).

Key Event Description

During vertebrate brain development, the gastrulation brain homeobox 2 gene (gbx2) is expressed in the forebrain (Z. Wang et al., 2018). The genes encoding the Gbx-type homeodomain transcription factors have been identified in a variety of vertebrates, and are primarily implicated in the regulation of various aspects of vertebrate brain development (Nakayama et al., 2017). Gbx2 exhibits DNA-binding transcription factor activity, RNA polymerase II-specific. Involved in cerebellum development; iridophore differentiation; and telencephalon regionalization. Predicted to localize to nucleus. Is expressed in several structures, including midbrain hindbrain boundary neural keel; midbrain hindbrain boundary neural rod; midbrain neural rod; nervous system; and presumptive rhombomere 1. Orthologous to human GBX2 (gastrulation brain homeobox 2) (*ZFIN Gene: Gbx2*, n.d.)

Retinoids such as retinoic acid (RA) are chemopreventive and chemotherapeutic agents. One source of RA is vitamin A, derived from dietary β-carotene. RA regulates cell proliferation, differentiation, and morphogenesis (X. J. Wang et al., 2007). It inhibits tumorigenesis through suppression of cell growth and stimulation of cellular differentiation (Soprano et al., 2004). Also, RA promotes apoptosis (Atencia et al., 1997; Herget et al., 2000), and this property may contribute to its antitumor properties. The effects of retinoids are mediated by specific nuclear receptors, namely, retinoic acid receptors (RAR-α, -β, and -γ) and retinoid X receptors (RXR- α, - β, and - γ) (Rochette-Egly & Chambon, 2001). RXRs form heterodimers with RARs or other nuclear hormone receptors and function as transcriptional regulators. Retinoids can either activate or repress gene expression through RAR/RXR heterodimers interacting with other transcription factors, such as AP-1, estrogen receptor α, and NF-κB activities (Shaulian & Karin, 2002). Retinoic acid has been shown to repress Gbx2 expression in talencephalon in Zebrafish and other vertebrate models in early stages of development.

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List of Key Events in the AOP

Event: 1903: foxi1 expression, increased

Short Name: foxi1 expression, increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:410 - Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased	

mortality

AOP ID and Name | Key Event **Event Type**

Biological Context

Level of Biological Organization

Molecular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI

Sex Applicability

Sex	Evidence
Unspecific	High

Foxi I class genes have been described in zebrafish (Hans et al., 2004; Solomon et al., 2003), humans (Larsson et al., 1995; Pierrou et al., 1994), mouse (Hulander et al., 1998; Overdier et al., 1997), rat (Clevidence et al., 1993) and Xenopus (Lef et al., 1994, 1996). However, it is unclear whether zebrafish foxi1 is orthologous to any one of these genes. The Xenopus FoxI1c (Lef et al., 1996), FoxI1a and FoxI1b genes (Lef et al., 1994) share the highest degree of sequence conservation with the zebrafish gene. The expression pattern of the two Xenopus pseudoallelic variants FoxI1a/b does not suggest functional similarity to zebrafish foxi1. Of the three Xenopus FoxI genes, FoxI1c (XFD-10) is most similar to foxi1 in sequence. However, Xenopus FoxI1c was reported to be expressed in the neuroectoderm and somites but not in the otic placode, unlike the pattern for foxi1 reported in (Lef et al., 1996). (Pohl et al., 2002) report provides a more detailed description of Xenopus FoxI1c, which suggests that this gene is expressed in preplacodal tissue and the branchial arches, similar to observations for zebrafish foxi1. Thus, it appears probable that Xenopus FoxI1c represents the ortholog of zebrafish foxi1 (Solomon et al., 2003).

Key Event Description

Foxi1 exhibits DNA-binding transcription factor activity. Involved in several processes, including animal organ development; epidermal cell fate specification; and neuron development. Predicted to localize to nucleus. Is expressed in several structures, including ectoderm; epibranchial ganglion; head; neural crest; and neurogenic field. Human ortholog(s) of this gene implicated in autosomal recessive nonsyndromic deafness 4. Orthologous to human FOXI1 (forkhead box I1) (*ZFIN Gene: Foxi1*, n.d.). The zebrafish Foxi1 protein shares 52% identity with Xenopus FoxI1c and 40% with human FOXI1; the forkhead domains are 95% and 94% identical, respectively (Solomon et al., 2003).

Zebrafish Foxi1 is expressed in nonneural ectoderm. Based on double in situ labeling with otx2, the anterior-most region of foxi1 expression lies just posterior to the midbrain hindbrain boundary. At the three-somite stage, the two domains of foxi1 expression become more compact, but are still located in approximately the same position lateral to the hindbrain (Solomon et al., 2003).

How it is Measured or Detected

Inhibition of expression can be measured with reverse transcription polymerase chain reaction (RT-PCR). This technique is primarily used to measure the amount of specific RNA which is achieved by monitoring the amplification reaction using fluorescence, a technique called real-time PCR or quantitative PCR (qPCR) (Wong & Medrano, 2005). Combined RT-PCR and qPCR are routinely used for analysis of gene expression.

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ZFIN Gene: foxi1. (n.d.). Retrieved April 12, 2021, from https://zfin.org/ZDB-GENE-030505-1

Event: 1904: six1b expression, increased

Short Name: six1b expression, increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:410 - Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	KeyEvent

Biological Context

Level of Biological Organization

Molecular

Domain of Applicability

Evidence was provided for vertebrates ((Brodbeck & Englert, 2004; Heanue et al., 1999; Li et al., 2003; Wawersik & Maas, 2000) and Drosophila (Bui et al., 2000).

Key Event Description

Six1b is predicted to have DNA-binding transcription factor activity, RNA polymerase II-specific and RNA polymerase II cis-regulatory region sequence-specific DNA binding activity. Involved in several processes, including muscle organ development; nervous system development; and regulation of skeletal muscle cell proliferation. Human ortholog(s) of this gene implicated in autosomal dominant nonsyndromic deafness; branchiootorenal syndrome; and nephroblastoma. Orthologous to human SIX1 (SIX homeobox 1) (*ZFIN Gene: Six1b*, n.d.).

Six1b is a Member of the Pax–Six1b–Eya–Dach (paired box–sine oculis homeobox–eyes absent– dachshund) gene regulatory network, involved in the development of numerous organs and tissues (Bessarab et al., 2004; Bricaud et al., 2006). It has been proposed to play an important role in inner ear development (Baker & Bronner-Fraser, 2001; Whitfield et al., 2002). Six1b expression appears to be regulated by pax2b and also by foxi1 (forkhead box I1) as expected for an early inducer of the otic placode (Bricaud et al., 2006).

Six1b promotes hair cell fate and, conversely, inhibits neuronal fate by differentially affecting cell proliferation and cell death in these lineages. Gain/loss-of-function experiment results indicate that, when six1 is overexpressed, not only are fewer neural progenitors formed but many of these progenitors do not go on to differentiate into neurons (Bricaud et al., 2006).

How it is Measured or Detected

Inhibition of expression can be measured with reverse transcription polymerase chain reaction (RT-PCR). This technique is primarily used to measure the amount of specific RNA which is achieved by monitoring the amplification reaction using fluorescence, a technique called real-time PCR or quantitative PCR (qPCR) (Wong & Medrano, 2005). Combined RT-PCR and qPCR are routinely used for analysis of gene expression.

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ZFIN Gene: six1b. (n.d.). Retrieved April 12, 2021, from https://zfin.org/ZDB-GENE-040426-230

Event: 1905: eya1 expression, inhibited

Short Name: eya1 expression, inhibited

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:410 - Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	KeyEvent

Biological Context

Level of Biological Organization

Molecular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI

Evidence was provided zebrafish (Kozlowski et al., 2005), Drosophila and vertebrates (Li et al., 2003; Zimmerman et al., 1997), and human (Abdelhak et al., 1997)

Key Event Description

Eya1 is predicted to have protein tyrosine phosphatase activity. Involved in adenohypophysis development; otic vesicle morphogenesis; and otolith development. Predicted to localize to nucleus. Is expressed in several structures, including adenohypophyseal placode; brain; ectoderm; head; and lateral line system. Orthologous to human EYA1 (EYA transcriptional coactivator and phosphatase 1) (*ZFIN Gene: Eya1*, n.d.).

Eyes absent (Eya) genes regulate organogenesis in both vertebrates and invertebrates. Mutations in human EYA1 cause congenital Branchio-Oto-Renal (BOR) syndrome and hereditary syndromic deafness, while targeted inactivation of murine Eya1 impairs early developmental processes in multiple organs, including ear, kidney and skeletal system (Kozlowski et al., 2005; Xu et al., 2002).

In zebrafish, the eya1 gene is widely expressed in placode-derived sensory organs during embryogenesis. Eya1 function appears to be primarily required for survival of sensory hair cells in the developing ear and lateral line neuromasts (Kozlowski et al., 2005).

How it is Measured or Detected

Inhibition of expression can be measured with reverse transcription polymerase chain reaction (RT-PCR). This technique is primarily used to measure the amount of specific RNA which is achieved by monitoring the amplification reaction using fluorescence, a technique called real-time PCR or quantitative PCR (qPCR) (Wong & Medrano, 2005). Combined RT-PCR and qPCR are routinely used for analysis of gene expression

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ZFIN Gene: eya1. (n.d.). Retrieved April 12, 2021, from https://zfin.org/ZDB-GENE-990712-18

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Event: 1825: Increase, Cell death

Short Name: Increase, Cell death

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:264 - Uncoupling of oxidative phosphorylation leading to growth inhibition via increased cell death	KeyEvent
Aop:266 - Uncoupling of oxidative phosphorylation leading to growth inhibition via oxidative DNA damage	KeyEvent
Aop:267 - Uncoupling of oxidative phosphorylation leading to growth inhibition via increased lipid peroxidation	KeyEvent
Aop:268 - Uncoupling of oxidative phosphorylation leading to growth inhibition via increased protein oxidation	KeyEvent
Aop:291 - Mitochondrial ATP synthase antagonism leading to growth inhibition (2)	KeyEvent
Aop:287 - Mitochondrial complex III antagonism leading to growth inhibition (2)	KeyEvent
Aop:368 - Cytochrome oxidase inhibition leading to olfactory nasal lesions	KeyEvent
Aop:377 - Dysregulated prolonged Toll Like Receptor 9 (TLR9) activation leading to Acute Respiratory Distress Syndrome (ARDS) and Multiple Organ Dysfunction (MOD)	KeyEvent
Aop:410 - Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	KeyEvent
Aop:418 - Aryl hydrocarbon receptor activation leading to impaired lung function through AHR-ARNT toxicity pathway	KeyEvent

Stressors

Name
Food deprivation
Gentamicin

Biological Context

Level of Biological Organization

Cellular

Cell term

Cell term

cell

Organ term

Organ term

organ

Evidence for Perturbation by Stressor

Food deprivation

Autophagy can be initiated by a variety of stressors, most notably by nutrient deprivation (caloric restriction) or can result from signals present during cellular differentiation and embryogenesis and on the surface of damaged organelles (Mizushima et al., 2008).

Gentamicin

Gentamicin causes significant inner ear sensory hair cell death and auditory dysfunction in zebrafish (Uribe et al., 2013).

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

The process of cell death is highly conserved within multi-cellular organisms. (Lockshin & Zakeri, 2004).

Key Event Description

Cell death is part of normal development and maturation cycle, and is the component of many response patterns of living tissues to xenobiotic agents (i.e.. micro organisms and chemicals) and to endogenous modulations, such as inflammation and disturbed blood supply (Kanduc et al., 2002). Many physiological processes require cell death for their function (e.g.., embryonal development and immune selection of B and T cells) (Bertheloot et al., 2021). Defects in cells that result in their inappropriate survival or untimely death can negatively impact development or contribute to a variety of human pathologies, including cancer, AIDS, autoimmune disorders, and chronic infection. Cell death may also occur following exposure to environmental toxins or cytotoxic chemicals. Although this is often harmful, it can be beneficial in some cases, such as in the treatment of cancer (Crowley et al., 2016).

Cell death can be divided into: programmed cell death (cell death as a normal component of development) and non-programmed cell death (uncontrolled death of the cell). Although this simplistic view has blurred the intricate mechanisms separating these forms of cell death, studies have and will uncover new effectors, cell death pathways and reveal a more complex and intertwined landscape of processes involving cell death (Bertheloot et al., 2021).

Programmed cell death: is a form of cell death in which the dying cell plays an active part in its own demise (Cotter & Al-Rubeai, 1995).

Apoptosis At a morphological level, it is characterized by cell shrinkage rather than the swelling seen in necrotic cell death. It is characterized

by a number of characteristic morphological changes in the structure of the cell, together with a number of enzyme-dependent biochemical processes. The result of it being the clearance of cells from the body, with minimal damage to surrounding tissues. An essential feature of apoptosis is the release of cytochrome c from mitochondria, regulated by a balance between proapoptotic and antiapoptotic proteins of the BCL-2 family, initiator caspases (caspase-8, -9 and -10) and effector caspases (caspase-3, -6 and -7). Apoptosis culminates in the breakdown of the nuclear membrane by caspase-6, the cleavage of many intracellular proteins (e.g., PARP and lamin), membrane blebbing, and the breakdown of genomic DNA into nucleosomal structures (Bertheloot et al., 2021). Mechanistically, two main pathways contribute to the caspase activation cascade downstream of mitochondrial cytochrome c release:

- Intrinsic pathway is triggered by dysregulation of or imbalance in intracellular homeostasis by toxic agents or DNA damage. It is characterized by mitochondrial outer membrane permeabilization (MOMP), which results in the release of cytochrome c into the cytosol.
- Extrinsic pathway is initiated by activation of cell surface death receptors. Proapoptotic death receptors include TNFR1/2, Fas and the TNF-related apoptosis-inducing ligand (TRAIL) receptors DR4 and DR5.

Other pathways of programmed cell death are called »non-apoptotic programmed cell-death« or »caspase-independent programmed cell-death« (Blank & Shiloh, 2007).

Necroptosis: This type of regulated cell death, occurs following the activation of the tumor necrosis receptor (TNFR1) by TNFα. Activation of other cellular receptors triggers necroptosis. These receptors include death receptors (i.e., Fas/FasL), Toll-like receptors (TLR4 and TLR3) and cytosolic nucleic acid sensors such as RIG-I and STING, which induce type I interferon (IFN-I) and TNFα production and thus promote necroptosis in an autocrine feedback loop. Most of these pathways trigger NFκB- dependent proinflammatory and prosurvival signals.

Pyroptosis is a type of cell death culminating in the loss of plasma membrane integrity and induced by activation of so-called inflammasome sensors. These include the Nod-like receptor (NLR) family, the DNA receptor Absent in Melanoma 2 (AIM2) and the Pyrin receptor.

Autophagy: is a process where cellular components such as macro proteins or even whole organelles are sequestered into lysosomes for degradation (Mizushima et al., 2008; Shintani & Klionsky, 2004). The lysosomes are then able to digest these substrates, the components of which can either be recycled to create new cellular structures and/or organelles or alternatively can be further processed and used as a source of energy (D'Arcy, 2019).

Anoikis is apoptosis induced by loss of attachment to substrate or to other cells (anoikis). Anoikis overlaps with apoptosis in molecular terms, but is classified as a separate entity because of its specific form od induction (Blank & Shiloh, 2007). Induction of anoikis occurs when cells lose attachment to ECM, or adhere to an inappropriate type of ECM, the latter being the more relevant *in vivo* (Gilmore, 2005).

Cornification: is programmed cell death of keratinocytes. Cell death in the context of cornification involves distinct enzyme classes such as transglutaminases, proteases, DNases and others (Eckhart et al., 2013).

Non-programmed cell death: occurs accidentally in an unplanned manner.

Necrosis is generally characterized to be the uncontrolled death of the cell, usually following a severe insult, resulting in spillage of the contents of the cell into surrounding tissues and subsequent damage thereof (D'Arcy, 2019).

How it is Measured or Detected

Assays for Quantitating Cell Death:

- Cell death can be measured by staining a sample of cells with trypan blue, assay is described in protocol: Measuring Cell Death by Trypan Blue Uptake and Light Microscopy (Crowley, Marfell, Christensen, et al., 2015d). Or with propidium Iodide, assay is described in protocol: Measuring Cell Death by Propidium Iodide (PI) Uptake and Flow Cytometry (Crowley & Waterhouse, 2015a)
- TUNEL technique: in situ terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling can be used to detect apoptotic cells (Bever & Fekete, 1999; Uribe et al., 2013).

Assays for Quantitating Cell Survival

Colony-forming assay can be used to define the number of cells in a population that are capable of proliferating and forming large groups of cells. Described in Protocol: Measuring Survival of Adherent Cells with the Colony-Forming Assay (Crowley, Christensen, & Waterhouse, 2015c); Measuring Survival of Hematopoietic Cancer Cells with the Colony-Forming Assay in Soft Agar (Crowley & Waterhouse, 2015b).

ASSAYS TO DISTINGUISH APOPTOSIS FROM NECROSIS AND OTHER DEATH MODALITIES

Detecting Nuclear Condensation: The nucleus is generally round in healthy cells but fragmented in apoptotic cells. Dyes such as Giemsa or hematoxylin, which are purple in color and therefore easily viewed using light microscopy, are commonly used to stain the nucleus. Other features of apoptosis and necrosis, such as plasma membrane blebbing or rupture, can be identified by staining the cytoplasm with eosin. Eosin is pinkish in color and can also be viewed using light microscopy. Hematoxylin and eosin are, therefore, commonly used together to stain cells. Assay is described in Protocol: Morphological Analysis of Cell Death by Cytospinning Followed by Rapid Staining (Crowley, Marfell, & Waterhouse, 2015c); Analyzing Cell Death by Nuclear Staining with Hoechst 33342 (Crowley, Marfell, & Waterhouse, 2015a).

Detection of DNA Fragmentation: Apoptotic cells with fragmented DNA can be identified and distinguished from live cells by staining with Propidium Iodide (PI) and measuring DNA content by flow cytometry. This assay is described in Protocol: Measuring the DNA Content of Cells in Apoptosis and at Different Cell-Cycle Stages by Propidium Iodide Staining and Flow Cytometry (Crowley, Chojnowski, & Waterhouse, 2015a). **TUNEL technique** can also be used**:** in situ terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling can be used to

detect apoptotic cells (Bever & Fekete, 1999; Crowley, Marfell, & Waterhouse, 2015b; Uribe et al., 2013).

Detecting Phosphatidylserine Exposure: Apoptosis is also characterized by exposure of phosphatidylserine (PS) on the outside of apoptotic cells, which acts as a signal that triggers removal of the dying cell by phagocytosis. Annexin V, can selectively bind to PS to label apoptotic cells in which PS is exposed. Purified annexin V can be conjugated to various fluorochromes, which can then be visualized by fluorescence microscopy or detected by flow cytometry. This assay is described in protocol: Quantitation of Apoptosis and Necrosis by Annexin V Binding, Propidium Iodide Uptake, and Flow Cytometry (Crowley, Marfell, Scott, et al., 2015e).

Detecting Caspase Activity: antibodies that specifically recognize the cleaved fragments of caspases and their substrates can be used to specifically detect caspase activity in apoptotic cells by immunocytochemistry. Flow cytometry (using primary antibodies conjugated to fluorescent molecules, or by counter staining with fluorescently labeled antibodies against the primary antibody) can then be used to quantitate the number of apoptotic cells. This assay is described in protocol: Detecting Cleaved Caspase-3 in Apoptotic Cells by Flow Cytometry (Crowley & Waterhouse, 2015a).

Detecting Mitochondrial Damage: flow cytometry can be used to quantitate the number of cells that have reduced mitochondrial transmembrane potential, which is commonly associated with cytochrome c release during apoptosis. For this assay see protocol: Measuring Mitochondrial Transmembrane Potential by TMRE Staining (Crowley, Christensen, & Waterhouse, 2015b).

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Event: 1930: altered, inner ear development

Short Name: Altered, inner ear development

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:410 - Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	KeyEvent

Stressors

Name
Gentamicin

Biological Context

Level of Biological Organization

Organ

Organ term

Organ term

ear

Evidence for Perturbation by Stressor

Gentamicin

Aminoglycoside antibiotics, like gentamicin, kill inner ear sensory hair cells in a variety of species including chickens, mice, and humans. The zebrafish (Danio rerio) has been used to study hair cell cytotoxicity in the lateral line organs of larval and adult animals. To assess the ototoxic effects of gentamicin, adult zebrafish received a single 250 mg/kg intraperitoneal injection of gentamicin and, 24 hours later, auditory evoked potential recordings (AEPs) revealed significant shifts in auditory thresholds compared to untreated controls (Uribe *et al*., 2013).

Uribe, P. M. *et al*. (2013) 'Aminoglycoside-Induced Hair Cell Death of Inner Ear Organs Causes Functional Deficits in Adult Zebrafish (Danio rerio)', *PLoS ONE*, 8(3), p. 58755. doi: 10.1371/journal.pone.0058755.

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Embryo	High

Sex Applicability

Sex	Evidence
Unspecific	High

Evidence was provided for Zebrafish, Chick and Mouse (Whitfield, 2015)

Key Event Description

Zebrafish:

The zebrafish (Danio rerio), a genetically tractable vertebrate, lends itself particularly well as a model system in which to study the ear. Zebrafish do not possess outer or middle ears, but have a fairly typical vertebrate inner ear, the normal development and anatomy of which has been described in a series of atlas-type papers (Haddon and Lewis, 1996; Bang, Sewell and Malicki, 2001). Although the zebrafish ear does not contain a specialized hearing organ—there is no equivalent of the mammalian cochlea—many features are conserved with other vertebrate species (Whitfield, 2002).

Inner ear develops from an ectodermal thickening, the otic placode, visible on either side of the hindbrain from mid-somite stages. In the zebrafish, this placode cavitates to form a hollow ball of epithelium, the otic vesicle, from which all structures of the membranous labyrinth and the neurons of the statoacoustic (VIIIth) ganglion arise (Haddon and Lewis, 1996; Whitfield *et al.*, 2002).

The mature organ, found in all jawed vertebrates, has two functions: it serves as an auditory system, which detects sound waves, and as a vestibular system, which detects linear and angular accelerations, enabling the organism to maintain balance (Whitfield *et al.*, 1996).

How it is Measured or Detected

Zebrafish:

- Direct observation of internal anatomic structures of zebrafish embryos. Defects visible under the dissecting microscope (Whitfield, 2002)
- Comparison of swimming patterns with wild-type fish. *Dog-eared* embryos are less responsive to vibrational stimuli, fail to maintain balance when swimming, and may circle when disturbed, a behavior characteristic of fish with vestibular defects (Nicolson *et al.*, 1998)
- High-throughput behavioral screening method for detecting auditory response defects in zebrafish. Assay monitors a rapid escape reflex in response to a loud sound (Bang *et al.*, 2002).

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Event: 1008: Reduced, Hearing

Short Name: Reduced, Hearing

Key Event Component

Process	Object	Action
sensory perception of sound		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:410 - Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	KeyEvent

Biological Context

Level of Biological Organization

Organ

Organ term

Organ term

ear

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates		NCBI
Invertebrates	Invertebrates		NCBI

- A sense of hearing is known to exist in a wide range of vertebrates and invertebrates, although the organs and structures involved vary widely.

Key Event Description

Hearing refers to the ability to perceive sound vibrations propagated as pressure changes through a medium such as air or water. Reduced hearing in the context of this key event can refer to reduction in the perceived volume of a sound relative to the amplitude of sound waves. Reduced hearing may also refer to a reduced range of frequencies that can be perceived.

How it is Measured or Detected

Hearing is generally measured behaviorally or electrophysiologically.

- Common behavioral tests involve transmission of pure tones of defined amplitude and frequency using and audiometer or PC and using a behavioral response (e.g., clicking a button; startle response) to determine whether the tone is perceived.

Electrophysiological tests:

- Auditory brainstem response (ABR): Uses electrodes placed on the head to detect auditory evoked potentials from background electrical activity in the brain.

Hearing tests in Fish:

- Through the mid-late 1980s conditioning and behavioral tests were most commonly employed in testing fish hearing. Methods reviewed by Fay (1988)
- A high throughput behavioral test for detecting auditory response in fish has been described (Bang et al. 2002).
- Invasive electrophysiological methods involving surgical insertion of electrodes into the auditory nerves have been employed.
- Non-invasive recording of Auditory Evoked Potentials (AEPs; synonymous with ABRs) are now the most common approach for measuring hearing in fish. AEPs can be recorded via electrodes attached cutaneously to the head (see review by Ladich and Fay, 2013).

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Event: 351: Increased Mortality

Short Name: Increased Mortality

Key Event Component

Process	Object	Action
mortality		increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:16 - Acetylcholinesterase inhibition leading to acute mortality	AdverseOutcome
Aop:96 - Axonal sodium channel modulation leading to acute mortality	AdverseOutcome
Aop:104 - Altered ion channel activity leading impaired heart function	AdverseOutcome
Aop:113 - Glutamate-gated chloride channel activation leading to acute mortality	AdverseOutcome
Aop:160 - Ionotropic gamma-aminobutyric acid receptor activation mediated neurotransmission inhibition leading to mortality	AdverseOutcome
Aop:161 - Glutamate-gated chloride channel activation leading to neurotransmission inhibition associated mortality	AdverseOutcome
Aop:138 - Organic anion transporter (OAT1) inhibition leading to renal failure and mortality	AdverseOutcome
Aop:177 - Cyclooxygenase 1 (COX1) inhibition leading to renal failure and mortality	AdverseOutcome
Aop:186 - unknown MIE leading to renal failure and mortality	AdverseOutcome
Aop:312 - Acetylcholinesterase Inhibition leading to Acute Mortality via Impaired Coordination & Movement	AdverseOutcome
Aop:320 - Binding of viral S-glycoprotein to ACE2 receptor leading to acute respiratory distress associated mortality	AdverseOutcome
Aop:155 - Deiodinase 2 inhibition leading to increased mortality via reduced posterior swim bladder inflation	AdverseOutcome
Aop:156 - Deiodinase 2 inhibition leading to increased mortality via reduced anterior swim bladder inflation	AdverseOutcome
Aop:157 - Deiodinase 1 inhibition leading to increased mortality via reduced posterior swim bladder inflation	AdverseOutcome
Aop:158 - Deiodinase 1 inhibition leading to increased mortality via reduced anterior swim bladder inflation	AdverseOutcome
Aop:159 - Thyroperoxidase inhibition leading to increased mortality via reduced anterior swim bladder inflation	AdverseOutcome
Aop:363 - Thyroperoxidase inhibition leading to increased mortality via altered retinal layer structure	AdverseOutcome
Aop:377 - Dysregulated prolonged Toll Like Receptor 9 (TLR9) activation leading to Acute Respiratory Distress Syndrome (ARDS) and Multiple Organ Dysfunction (MOD)	AdverseOutcome
Aop:364 - Thyroperoxidase inhibition leading to increased mortality via decreased eye size	AdverseOutcome
Aop:365 - Thyroperoxidase inhibition leading to increased mortality via altered photoreceptor patterning	AdverseOutcome
Aop:399 - Inhibition of Fyna leading to increased mortality via decreased eye size (Microphthalmos)	AdverseOutcome
Aop:413 - Oxidation and antagonism of reduced glutathione leading to mortality via acute renal failure	AdverseOutcome
Aop:410 - Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	KeyEvent

Biological Context

Level of Biological Organization

Population

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
all species	all species	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	Moderate

All living things are susceptible to mortality.

Key Event Description

Increased mortality refers to an increase in the number of individuals dying in an experimental replicate group or in a population over a specific period of time.

How it is Measured or Detected

Mortality of animals is generally observed as cessation of the heart beat, breathing (gill or lung movement) and locomotory movements. Mortality is typically measured by observation. Depending on the size of the organism, instruments such as microscopes may be used. The reported metric is mostly the mortality rate: the number of deaths in a given area or period, or from a particular cause.

Depending on the species and the study setup, mortality can be measured:

- in the lab by recording mortality during exposure experiments
- in dedicated setups simulating a realistic situation such as mesocosms or drainable ponds for aquatic species
- in the field, for example by determining age structure after one capture, or by capture-mark-recapture efforts. The latter is a method commonly used in ecology to estimate an animal population's size where it is impractical to count every individual.

Regulatory Significance of the AO

Increased mortality is one of the most common regulatory assessment endpoints, along with reduced growth and reduced reproduction.

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 2485: GSK3beta inactivation leads to Repression of Gbx2 expression

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	adjacent	High	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebra fish	Danio rerio	High	NCBI
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Evidence for this KER is provided for zebrafish (Wang *et al.*, 2018) and humans (Grassilli *et al.*, 2014; Kim *et al.*, 2018).

Key Event Relationship Description

Wnt signaling is implicated in anteroposterior (AP) axis patterning and midbrain specification in both animal and human systems. GSK3 is a key enzyme mediating the canonical Wnt signaling. BIO a known GSK3 inhibitor activates canonical Wnt signal pathway. Gbx2 is one of the representative AP markers (Kim *et al.*, 2018).

Evidence Supporting this KER

Biological Plausibility

- Zebrafish embryos were treated with chemical inhibitors or activators of various signaling pathways, such as the Wnt, FGF, retinoic acid (RA), HH, BMP, Nodal, and Notch pathways, from 14 hpf to 18 hpf, immediately before the advent of gbx2 expression in the telencephalon, and than gbx2 expression was examined in the telencephalon. In embryos treated with BIO, a selective GSK3 inhibitor that activates Wnt signaling (Sato et al., 2004), gbx2 expression was specifically repressed in the telencephalon, but was unaffected or weakly activated in the isthmus and OV (Wang *et al.*, 2018).
- Treatment of human ESC-derived NCPs with BIO (Gsk3b inhibitor) downregulated expression of GBX2 in dose dependent manner (Kim *et al.*, 2018). Quantitative gene expression analysis following seven days of treatment revealed that the GBX2 expression decreased as the BIO concentration increased (Kim *et al.*, 2018).
- To confirm whether the effect of BIO on midbrain specification was indeed through the activation of canonical Wnt signal, other small molecules that inhibit GSK3 were tested in different modes of action, such as 1- AKP and LiCl on human ESC-derived NPCs. LiCl treatment elicited similar gene expression patterns (decreased expression of GBX2) as BIO treatment, although the fold changes in gene expression were lower than those of the other inhibitors. These data support that midbrain-specific gene expression results from the activation of canonical Wnt signal via GSK3 inhibition (Kim *et al.*, 2018).

Empirical Evidence

No Data.

Uncertainties and Inconsistencies

No Data.

Quantitative Understanding of the Linkage

No Data.

Response-response relationship

No Data.

Time-scale

Gbx2 begins to express in telencephalon approximately 14-18hpf (Wang *et al.*, 2018).

Known modulating factors

No Data.

Known Feedforward/Feedback loops influencing this KER

No Data.

References

Grassilli, E. *et al.* (2014) 'GSK3A is redundant with GSK3B in modulating drug resistance and chemotherapy-induced necroptosis', *PLoS ONE*, 9(7), pp. 1–8. doi: 10.1371/journal.pone.0100947.

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Wang, Z. *et al.* (2018) 'The role of gastrulation brain homeobox 2 (gbx2) in the development of the ventral telencephalon in zebrafish embryos', *Differentiation*, 99(December 2017), pp. 28–40. doi: 10.1016/j.diff.2017.12.005.

Relationship: 2436: Repression of Gbx2 expression leads to foxi1 expression, increased

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	adjacent	Moderate	Not Specified

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Larvae	Moderate

Sex Applicability

Sex	Evidence
Unspecific	Not Specified

The gastrulation brain homebox (Gbx) group of transcription factor genes, composed of two genes, gbx1 and gbx2, in vertebrates, is also present in invertebrates (Chiang et al., 1995), and can be regarded as widely conserved among animals (Wang et al., 2018). Gbx2 functions in a variety of developmental processes after midbrain-hindbrain boundary (MHB) establishment. (Burroughs-Garcia et al., 2011) data demonstrate that the role of gbx2 in anterior hindbrain development is functionally conserved between zebrafish and mice. This gene was shown to be required for neural crest (NC) formation in mice (B. Li et al., 2009; Roeseler et al., 2012). In Xenopus gbx2 is the earliest factor for specifying neural crest (NC) cells, and that gbx2 is directly regulated by NC inducing signaling pathways, such as Wnt/β-catenin signaling (Li et al., 2009).

Foxi I class genes have been described in zebrafish(Hans et al., 2004; Solomon et al., 2003), humans (Larsson et al., 1995; Pierrou et al., 1994), mouse (Hulander et al., 1998; Overdier et al., 1997), rat (Clevidence et al., 1993) and Xenopus (Lef et al., 1994, 1996). However, it is unclear whether zebrafish foxi1 is orthologous to any one of these genes. The Xenopus FoxI1c (Lef et al., 1996), FoxI1a and FoxI1b genes (Lef et al., 1994) share the highest degree of sequence conservation with the zebrafish gene. The expression pattern of the two Xenopus pseudoallelic variants FoxI1a/b does not suggest functional similarity to zebrafish foxi1. Of the three Xenopus FoxI genes, FoxI1c (XFD-10) is most similar to foxi1 in sequence. However, Xenopus FoxI1c was reported to be expressed in the neuroectoderm and somites but not in the otic placode, unlike the pattern for foxi1 reported in (Lef et al., 1996). (Pohl et al., 2002) report provides a more detailed description of Xenopus FoxI1c, which suggests that this gene is expressed in preplacodal tissue and the branchial arches, similar to observations for zebrafish foxi1. Thus, it appears probable that Xenopus FoxI1c represents the ortholog of zebrafish foxi1 (Solomon et al., 2003).

Key Event Relationship Description

Repression of Gbx2 expression leads to increased expression of foxi1.

Evidence Supporting this KER

Gbx2 exhibits DNA-binding transcription factor activity, RNA polymerase II-specific. Involved in cerebellum development; iridophore differentiation; and telencephalon regionalization. Predicted to localize to nucleus. Is expressed in several structures, including midbrain hindbrain boundary neural keel; midbrain hindbrain boundary neural rod; midbrain neural rod; nervous system; and presumptive rhombomere 1

(*ZFIN Gene: Gbx2*, n.d.). After MHB establishment, murine gbx2 expression continues in the anterior hindbrain, suggesting later developmental roles for this gene. Li et al. (2002) showed different requirements for gbx2 in cerebellum formation depending on the loci along the mediolateral axis (J. Y. H. Li et al., 2002). In zebrafish, gbx2 expression persists in the isthmus until at least the hatching stage (Kikuta et al., 2003), and the roles of gbx2 are conserved in the developing anterior hindbrain, including nV cranial motor neurons, in zebrafish and mice (Burroughs-Garcia et al., 2011).

A number of studies have shown that Gbx2 represses many developmental regulatory genes during MHB development including foxi1b (Nakamura, 2001; Rhinn & Brand, 2001; Simeone, 2000). Thus, Gbx2 may be a multifunctional transcriptional factor, although the mechanisms of the differential regulation of its activity during development are unknown (Nakayama et al., 2017). In (Nakayama et al., 2017) study Gbx2 has been shown to downregulate Foxi1 in zebrafish embryos.

Foxi1 exhibits DNA-binding transcription factor activity. Involved in several processes, including animal organ development; epidermal cell fate specification; and neuron development. Predicted to localize to nucleus. Is expressed in several structures, including ectoderm; epibranchial ganglion; head; neural crest; and neurogenic field (*ZFIN Gene: Foxi1*, n.d.).

Biological Plausibility

Foxi1 is one of the downstream genes regulated by gbx2 transcription factor. Downregulation of gbx2 leads to increased foxi1 expression in zebrafish embryos.

- (Nakayama et al., 2017) sought to comprehensively identify the target genes of zebrafish gbx2 at the end of gastrulation by microarray analysis. Eight genes that had been shown by the microarray data to be downregulated (Group C, otx1b, otx2, hoxb5b, msi2b, neurog1; Group D, pou5f3; Group F, her5, foxi1) were indeed immediately downregulated in hsp-gbx2+ embryos. Most of the genes that were identified as upregulated or downregulated in the microarray analysis were confirmed by qPCR analysis. WISH (whole mount in situ hybridization) further confirmed the alterations in expression for 6 out of the 12 genes examined (otx2, otx1b, her5, hesx1, klf2a, and pou5f3). Failure to detect the expression alterations of the remaining genes with WISH is likely due to the non-quantitative nature of the WISH technique, which can only detect marked differences in expression levels. It is additionally possible that gbx2 induction affected broad and low-level expression that was undetectable by their conventional WISH technique. Still, the qPCR and WISH results together confirmed the reliability of the comprehensive microarray analysis (Nakayama et al., 2017).

Empirical Evidence

No Data

Uncertainties and Inconsistencies

Failure to detect the expression alterations of the remaining genes with WISH is likely due to the non-quantitative nature of the WISH technique, which can only detect marked differences in expression levels. It is additionally possible that gbx2 induction affected broad and low-level expression that was undetectable by their conventional WISH technique. Still, the qPCR and WISH results together confirmed the reliability of the comprehensive microarray analysis (Nakayama et al., 2017).

Quantitative Understanding of the Linkage

No Data

Response-response relationship

No Data

Time-scale

(Wang et al., 2018) have shown that gbx2 is expressed in zebrafish (Danio rerio) embryos only after the late gastrula stage in the anterior hindbrain.

Known modulating factors

No Data

Known Feedforward/Feedback loops influencing this KER

No Data

References

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Relationship: 2437: foxi1 expression, increased leads to six1b expression, increased

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	adjacent	Moderate	Not Specified

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Embryo	High

Sex Applicability

Sex	Evidence
Unspecific	High

Data was provided for zebrafish (Bricaud et al., 2006; Lleras-Forero & Streit, 2012), mice and chick (Hulander et al., 2003; Lleras-Forero & Streit, 2012)

Key Event Relationship Description

Increased foxi1 expression leads to increased six1b expression.

Evidence Supporting this KER

The forkhead family member, foxi1 is an important player not only in the induction of the otic placode (Solomon et al., 2003) but also in the proper activation of differentiation pathways in the inner ear (Hans et al., 2004). Foxi1 transcription factor regulate *six* and *eya* gene expression during anamniote preplacodal induction. When foxi1 is knocked down, the ear anlagen is either entirely missing or greatly reduced (Solomon et al., 2003) and no expression of six1b is detectable (Bricaud et al., 2006). With loss-of-function experiment (Bricaud et al., 2006) demonstrated that foxi1 can regulate, directly or indirectly, six1b transcription in developing zebrafish inner ear. Six1b acts early in both hair cell and neuronal lineages. When six1b is overexpressed, not only are fewer neural progenitors formed but many of these progenitors do not go on to differentiate into neurons. Gain-of-function, together with the six1b loss-of-function results, suggest that six1b is necessary and sufficient for the normal formation of hair cells in the anterior macula, although it inhibits neuronal fate in the developing inner ear (Bricaud et al., 2006).

Biological Plausibility

Foxi1 is an early inducer of the otic placode and positively regulates the expression of six1b transcription factor.

- When foxi1 is knocked down, the ear anlagen is either entirely missing or greatly reduced (Solomon et al., 2003) and no expression of six1b is detectable in otocyst. Because, at 28 hpf, the lack of six1b expression could be secondary to the overall absence of the otic placode attributable to foxi1 loss-of-function, six1b expression was studied at either 28 hpf in embryos with less severe phenotype or at 16.5 hpf when the placode just arises. In both cases, no expression of six1b was detected (Bricaud et al., 2006).
- Overexpression of six1b during inner development was achieved by injecting a synthetic six1b mRNA at early stages. Such gain-of-function experiments gave the opposite phenotype to that seen after six1b loss-of-function. At 3 dpf, more hair cells are present. This overproduction of hair cells is detectable as early as 28 hpf, with an average of four hair cells observed instead of the two in wild-type embryos. We assayed for the presence of differentiated neurons at 3 dpf and neural precursors at 32 hpf with the neuronal markers HuC and neuroD, respectively. At 32 hpf in the six1b overexpressing embryos, fewer neuroD positive cells are detectable in the otic ganglion than in control embryos, suggesting that fewer neural progenitors are formed when six1b is overexpressed. At 3 dpf, the decrease in number of SAG neurons versus controls is even more dramatic. In extreme cases, SAG neurons are completely eliminated. These results indicate that, when six1b is overexpressed, not only are fewer neural progenitors formed but many ofthese progenitors do not go on to differentiate into neurons. In conclusion, these, together with the six1b loss-of-function results, suggest that six1b is necessary and sufficient for the normal formation of hair cells in the anterior macula, although it inhibits neuronal fate in the developing inner ear (Bricaud et al., 2006).

Empirical Evidence

No Data.

Uncertainties and Inconsistencies

Foxi1 gene is critical for zebrafish otic induction (Solomon et al., 2003), while it is not essential for this process in mice (Hulander et al., 2003).

Quantitative Understanding of the Linkage

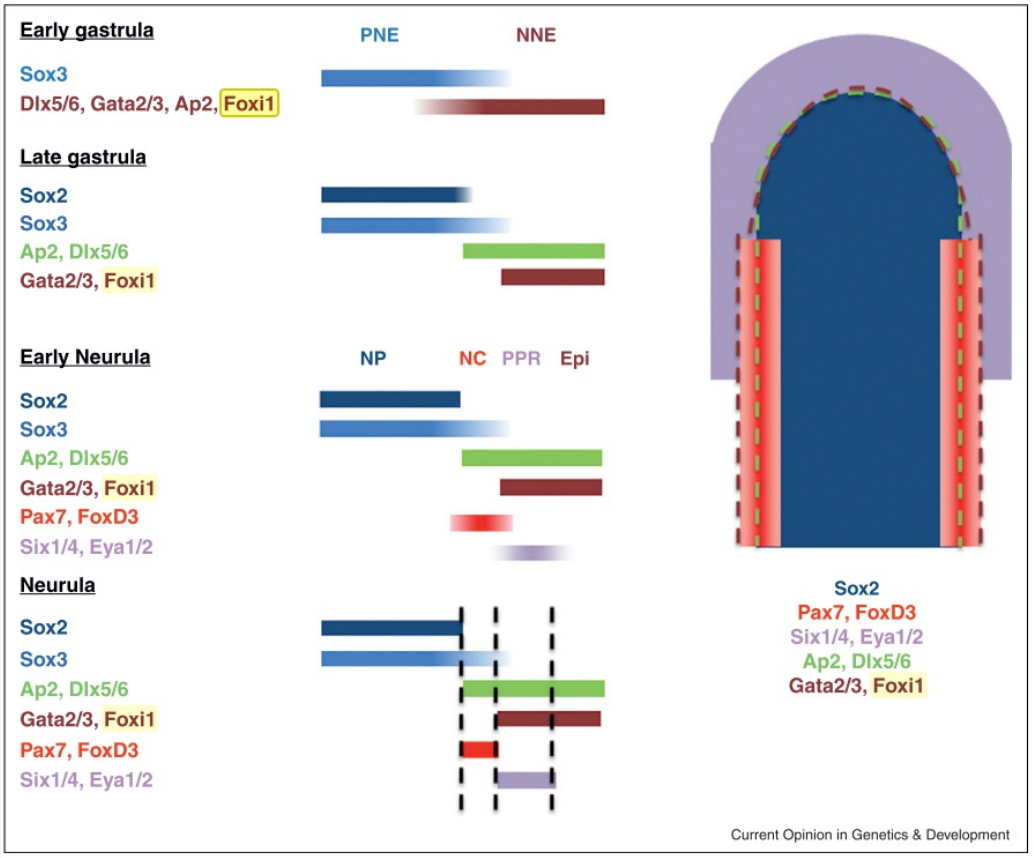
No Data.

Response-response relationship

No Data.

Time-scale

- **Expression of zebrafish six1b in the Inner Ear and Neuromasts:** Expression of six1b was observed in the developing inner ear and neuromasts of the lateral line until 96 hpf, the latest stage analyzed in this study. Transcripts of six1b were detected in all five sensory patches of the inner ear as well as in the semicircular canals. Detected first at 48 hpf, six1b expression in neuromasts of the midbody lateral line reached its peak at 72 hpf with stronger staining at the basal region of the neuromast, where bodies of hair cells are localized (Webb & Shirey, 2003).
- **Expression of zebrafish six1b in Muscles:** Since the beginning of segmentation six1b was expressed in the somites. At 72 hpf, the expression of six1b became more pronounced in the ventral somites with stronger staining in the most ventral cells. It continued in the pectoral fin and ventral abdomen muscle. Six1b expression was also found in the muscles of the eye and the lower jaw. (Bricaud et al., 2006).
- Temporal changes in gene expression and the emergence of sensory placode progenitors. As development proceeds gene expression domains sharpen through mutually repressive interactions; in the head region, the neural crest and placode precursor specific transcripts begin to be expressed at early neurula stages. Initially their boundaries are fuzzy, but gene expression resolves to distinct domains by late neurula (black dashed lines). NP: neural plate; NC: neural crest; PPR: preplacodal region; Epi: future epidermis. Right: diagram of an embryo at early neurula stages; dashed lines indicate the medial boundaries of non-neural transcripts (Lleras-Forero & Streit, 2012).



Known modulating factors

No Data.

Known Feedforward/Feedback loops influencing this KER

No Data.

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Relationship: 2438: six1b expression, increased leads to eya1 expression, inhibited

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	adjacent	Moderate	Not Specified

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Embryo	High

Sex Applicability

Sex	Evidence
Unspecific	High

Key event relationship described herein has been mostly studied on zebrafish model (Bessarab et al., 2004; Bricaud et al., 2006). Evidence was also provided for *Xenopus* (Bever & Fekete, 1999; Kil & Collazo, 2001), *Drosophila* (Brodbeck & Englert, 2004; Heanue et al., 1999; Li et al., 2003), mouse (Brodbeck & Englert, 2004; Li et al., 2003)

Key Event Relationship Description

Increase of six1b expression leads to inhibition of eya1.

Evidence Supporting this KER

Retinoic acid is required for both, expression of preplacodal ectoderm (PPE) markers Six1b and Eya1 and for the definition of their posterior boundary of expression (Schlosser, 2014). Six1b and Eya1 are not only expressed in otic placodes, but initially mark the whole preplacodal region (PPR) (Aghaallaei et al., 2007; Litsiou et al., 2005; Schlosser, 2006). Six1b expression appears to be regulated by pax2b and also by foxi1 (forkheadbox I1) as expected for an early inducer ofthe otic placode (Bricaud et al., 2006). In the inner ear, six1b expression is restricted to the ventral otocyst in which the first hair cells differentiate and prospective SAG neurons delaminate. six1b promotes formation of hair cells by increasing cell proliferation and independently inhibits neuronal development by inducing apoptosis (Bessarab et al., 2004; Bricaud et al., 2006). In zebrafish, the eya1 gene is widely expressed in placode-derived sensory organs during embryogenesis but Eya1 function appears to be primarily required for survival of sensory hair cells in the developing ear and lateral line neuromasts (Kozlowski et al., 2005). Eya and Six together with the Dach protein directly interact to form a functional transcription factor. In this complex, the DNA binding function is provided by the Six protein, Eya mediates transcriptional activation and Dach proteins appear to function as cofactors (López-Ríos et al., 2003). A regulatory network of these proteins is thought to be active also during ear development (Whitfield et al., 2002) and vertebrate eye development (Wawersik & Maas, 2000).

Biological Plausibility

Six1b is a transcription factor which inhibits expression of eya1.

- RT-PCR analysis first detected six1b mRNA at mid-gastrula and its expression level increased at the beginning of segmentation, when in situ hybridization first detected regionalized expression. Shortly after the tail bud stage, weak expression was observed in the horseshoe-shaped domain surrounding the anterior neural plate, corresponding to position of the cranial placode. During the segmentation period, expression of six1 was observed in the olfactory placode and in the region that later give rise to the otic vesicle as well as anterior and posterior lateral line placodes. These elements of expression resemble the patterns reported for zebrafish eya1 (Bessarab et al., 2004; Sahly et al., 1999)
- A regulatory network of DNA binding Six protein, eya1 transcriptional activator and Dach protein as cofactor is thought to be active during ear development (Whitfield et al., 2002) and vertebrate eye development (Wawersik & Maas, 2000).
- Six1b gain-of-function experiment results showed that overexpression of six1b in zebrafish developing inner ear inhibited expression of

eya1 (Bricaud et al., 2006).

- Catalytically active phosphatase Eya1 in vertebrates cooperates with the DNA-binding protein Six1 to promote gene induction in response to sonic hedgehog (Shh) signaling and Eya1/Six1 together regulate Gli transcriptional activators (Eisner et al., 2015; Whitfield et al., 2002).

Empirical Evidence

No Data

Uncertainties and Inconsistencies

- Interactions between Six1b and other members ofthe Pax–Six–Eya–Dach gene network, such as Eya1, also seem to differ between mouse and zebrafish. Zebrafish six1b inhibits eya1 expression, although its own expression is independent of the function of eya1. In mouse, Eya1 positively regulates Six1b expression (Xu et al., 1999), although its own expression is Six1b independent (Li et al., 2003; Zheng et al., 2003). Not only may interactions between six1b and eya1 differ in zebrafish relative to mouse but so might the interactions between six1b and the pax2 genes.
- six1b function seems restricted to the otic ganglia even though it is expressed in other ganglia. However,we cannot rule out more subtle effects of six1b in other cranial ganglia, such as controlling the type of receptors or neurotransmitters expressed by these neurons. The neural crest contribution to other placodes (Baker & Bronner-Fraser, 2001) could also make six1b function less obvious than in the SAG.

Quantitative Understanding of the Linkage

No Data.

Response-response relationship

No Data.

Time-scale

Six1b acts early in both hair cell and neuronal lineages. The lack of suitable markers for hair cell or SAG neuronal precursors means that assaying the identity of the dividing cells before they actually differentiate is currently not possible. Latest time point for six1b loss or gain-of-function rescue seems to be 15-48 hpf (Bricaud et al., 2006) which coicides with the initial wave of hair cell and neurnoal differentiation between 24-48 hpf observed during inner ear development (Haddon & Lewis, 1996).

Known modulating factors

No Data.

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Relationship: 2439: eya1 expression, inhibited leads to Increase, Cell death

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	adjacent	Moderate	Not Specified

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Embryo	High

Sex Applicability

Sex	Evidence
Unspecific	High

Evidence was provided for zebrafish (Kozlowski et al., 2005; Sahly et al., 1999), other vertebrates and Drosophila (Li et al., 2003; Zimmerman et al., 1997) and mammals (Li et al., 2003).

Key Event Relationship Description

Zebrafish Eya1 has a role in regulating apoptosis within developing otic vesicle. In mammals Eya1 dephosphorylates histone variant H2AX and thereby affects DNA repair and cell survival (Cook et al., 2009).

Evidence Supporting this KER

Zebrafish eya1 has a role in development of the cristae, statoacoustic ganglia, and lateral line system. Primary consequence of loss of eya1 function in the zebrafish embryo is premature apoptosis in precursors to these structures. Apoptosis has also resulted from loss of eya gene function in Drosophila and mouse (Bonini et al., 1993; Xu et al., 1999), these findings may reflect a general mechanism of suppression of apoptosis by Eya proteins. Evidence also indicates a role of Eya protein in regulating genes controlling precursor cell proliferation and survival during mammalian organogenesis (Li et al., 2003).

Biological Plausibility

Zebrafish Eya1 has a role in regulating apoptosis within developing otic vesicle. In mammals Eya1 dephosphorylates histone variant H2AX and thereby affects DNA repair and cell survival (Cook et al., 2009).

- Increased levels of apoptosis occur in the migrating primordia of the posterior lateral line in *dog* (the zebrafish mutation *dog-eared* that is defective in formation of the inner ear and lateral line sensory systems) embryos and as well as in regions of the developing otocyst that are mainly fated to give rise to sensory cells of the cristae. Because of the large number of apoptotic cells observed within the otic vesicle of *dog* mutants, it has been proposed that eya1 could act as a suppressor of apoptosis (Kozlowski et al., 2005). Eya1 could be required to prevent apoptosis in the hair cell lineage, whereas it could have opposite actions in the neuronal lineage (Bricaud et al., 2006).
- With loss of eya1 function in the eye primordium of *Drosophila*, the eye progenitor cells die by programmed cell death early in the differentiation process (Sahly et al., 1999).
- Ectopic cell death in the developing otic vesicle is not restricted to prospective crista cells in the lateral wall. Acridine orange staining of *dog* embryos and wild-type siblings at several times during development revealed that cell death can occur throughout the *dog* otic vesicle. Ectopic cell death throughout the otic vesicle is the likely cause of the smaller otic vesicles observed in *dog* embryos during embryogenesis (Kozlowski et al., 2005).
- By 55 hpf, the expression of crista-specific genes is severely reduced or absent in *dog* embryos and crista sensory hair cell bundles are absent at 72 hpf, suggesting that they have failed to differentiate (Whitfield et al., 2002).

Empirical Evidence

No Data.

Uncertainties and Inconsistencies

No Data.

Quantitative Understanding of the Linkage

No Data.

Response-response relationship

No Data.

Time-scale

Zebrafish morphological defects of the otic vesicle are first obvious at 48 hpf, some 38 h after the onset of eya1 expression in the preplacodal domain, and 24 h after increased apoptosis is observed. By 48 hpf, otic vesicles of the weakest *dog* phenotypic class are slightly smaller and more oblong in shape than wild-type siblings. As the phenotypic severity increases, *dog* otic vesicles are less round at the anterior end, developing an indented or folded appearance. By 72 hpf, *dog* otic vesicles are visibly smaller than those of wild-type siblings and distortion of the anterior end of the vesicle is more pronounced. At 96 hpf, otic vesicles of the severe phenotypic class are significantly smaller than wild-type siblings and have a narrow, cylindrical appearance (Kozlowski et al., 2005).

Known modulating factors

No Data.

Known Feedforward/Feedback loops influencing this KER

No Data.

References

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Relationship: 2467: Increase, Cell death leads to Altered, inner ear development

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	adjacent	Moderate	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Embryo	High

Sex Applicability

Sex	Evidence
Unspecific	High

Evidence was provided for Zebrafish (Whitfield *et al.*, 1996; Kozlowski *et al.*, 2005), other vertebrates (Schlosser *et al.*, 2008), mice (Johnson *et al.*, 1999; Xu *et al.*, 1999) and human (Bonini, Leiserson and Benzer, 1993).

Key Event Relationship Description

Increased cell death in otic vesicle leads to abnormal inner ear development.

Evidence Supporting this KER

The vertebrate inner ear develops from the otic placode, an ectodermal thickening that appears early in development and invaginates to form the otic vesicle (Aghaallaei et al., 2007). Eya1 gene was shown to regulate cell death during development of otic vesicle (Abdelhak et al., 1997; Kozlowski et al., 2005; Schlosser, 2014; Whitfield et al., 2002; Zhou et al., 2017). Increased cell death resulted in smaller otic vesicle (Kozlowski et al., 2005).

Biological Plausibility

Increased cell death in otic vesicle leads to sensory defects via malformations of inner ear and lateral line sensory systems (Kozlowski et al., 2005).

- Increased levels of apoptosis occur in the migrating primordia of the posterior lateral line in *dog* (the zebrafish mutation *dog-eared* that is defective in formation of the inner ear and lateral line sensory systems) embryos and as well as in regions of the developing otocyst that are mainly fated to give rise to sensory cells of the cristae. Ectopic cell death throughout the otic vesicle is the likely cause of the smaller otic vesicles observed in *dog* embryos during embryogenesis (Kozlowski et al., 2005).
- After Six1 or Eya1 loss of function, the numbers of sensory receptors and neurons in the sense organs and ganglia derived from the olfactory, otic, lateral line, profundal/trigeminal, and epibranchial placodes are reduced, and only small, malformed sense organs develop that are abnormally patterned and functionally deficient (Schlosser, 2014).
- Other cell types of the inner ear, including supporting cells and endolymph-producing cells, are also derived from the otic placode as are the sensory neurons of the vestibulocochlear ganglion, which innervate the hair cells. The lateral line placodes of fishes and amphibians also give rise to hair cells and supporting cells, which form small mechanosensory organs (neuromasts) distributed in lines along the body surface and involved in the detection of water movements. They also produce the sensory neurons innervating these receptor organs (Schlosser, 2014; Whitfield, 2002).
- *Dog-eared* zebrafish mutants exibit increased death in otic vesicle during development; loss of cristae; abnormal macuae and semicircular canal system (Kozlowski et al., 2005; Whitfield et al., 1996, 2002). Dog-eared mutants are zebrafish model for human branchio-oto renal syndrome (Whitfield, 2002).
- BOR (branchio-oto-renal) syndrome in humans is characterized by branchial cleft abnormalities, otic developmental defects and renal malformations. To date, autosomal dominant mutations in the EYA1 (Eyes Absent 1) gene are the most common genetic cause of BOR. EYA1 is the human homologue of the Drosophila gene eya (eyes absent), in which null mutations result in eyeless fly embryos due to apoptotic loss of eye disc cells (Bonini et al., 1993). Subsequent studies reported homologues of the eya gene in vertebrates (Duncan et al., 1997; Li et al., 2010).

Empirical Evidence

No Data.

Uncertainties and Inconsistencies

No Data.

Quantitative Understanding of the Linkage

No Data.

Response-response relationship

No Data.

Time-scale

Zebrafish morphological defects of the otic vesicle are first obvious at 48 hpf, some 38 h after the onset of eya1 expression in the preplacodal domain, and 24 h after increased apoptosis is observed. By 48 hpf, otic vesicles of the weakest *dog* phenotypic class are slightly smaller and more oblong in shape than wild-type siblings. As the phenotypic severity increases, *dog* otic vesicles are less round at the anterior end, developing an indented or folded appearance. By 72 hpf, *dog* otic vesicles are visibly smaller than those of wild-type siblings and distortion of the anterior end of the vesicle is more pronounced. At 96 hpf, otic vesicles of the severe phenotypic class are significantly smaller than wild-type siblings and have a narrow, cylindrical appearance (Kozlowski et al., 2005).

Known modulating factors

No Data.

Known Feedforward/Feedback loops influencing this KER

No Data.

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Relationship: 2468: Altered, inner ear development leads to Reduced, Hearing

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	adjacent	High	Low

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Unspecific	High

Key event relationship is applicable to wide range of vertebrates (Whitfield, 2015).

Key Event Relationship Description

The inner ear is the vertebrate organ of hearing and balance (Whitfield, 2002).

Evidence Supporting this KER

Inner ear develops from an ectodermal thickening, the otic placode, visible on either side of the hindbrain from mid-somite stages. In the zebrafish, this placode cavitates to form a hollow ball of epithelium, the otic vesicle, from which all structures of the membranous labyrinth and the neurons of the statoacoustic (VIIIth) ganglion arise (Haddon and Lewis, 1996; Whitfield *et al.*, 2002).

Biological Plausibility

Zebrafish serves as a model organism for hearing and deafness. Mutations in several genes connected to development of inner ear affect morphology and patterning of the inner ear epithelium, including formation of the semicircular canals and, in some, development of sensory patches (maculae and cristae). Zebrafish mutant embryos fail to balance correctly, and may swim on their sides, upside down, or in circles (Whitfield *et al.*, 1996). This is reminiscent of the behavior of deaf mouse mutants, which often display hyperactive circling or head bobbing due to vestibular dysfunction (Whitfield, 2002).

- Dog-*eared* mutants show abnormal development of semicircular canals and lack cristae within the ear (Kozlowski *et al.*, 2005), while in *van gogh*, semicircular canals fail to form altogether, resulting in a tiny otic vesicle containing a single sensory patch. Both mutants show irregular swimming pattern (Whitfield *et al.*, 1996).

Empirical Evidence

No Data.

Uncertainties and Inconsistencies

No Data.

Quantitative Understanding of the Linkage

No Data.

Response-response relationship

No Data.

Time-scale

No Data.

Known modulating factors

No Data.

Known Feedforward/Feedback loops influencing this KER

No Data.

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Relationship: 2231: Reduced, Hearing leads to Increased Mortality

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Repression of Gbx2 expression leads to defects in developing inner ear and consequently to increased mortality	adjacent	High	High

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Low

Sex Applicability

Sex	Evidence
Unspecific	Low

Key Event Relationship Description

Impaired hearing could result in an impact on ecologically relevant endpoint, such as predator avoidance and prey capture. Therefore, it can be assumed that an affect on hearing could reduce young of year survival.

Evidence Supporting this KER

Biological Plausibility

- In birds, acoustic signals play key roles in territory defense and mate attraction (Slabbekoorn and Ripmeester, 2008).

Roles of Acoustic signaling in fish (reviewed by Kasumayan 2009):

- Reproductive isolation - among fish capable of generating sound, sound emission during spawning is the most prominent life stage during which acoustic signaling occurs. Includes mate attraction, courtship, establishment of territory.
- Defensive sounds - fright and stress, alert conspecifics to potential threats.
- Organization of group/aggregative behaviors
- Feeding behaviors - in many fish conditioned reflex to the sounds of conspecifics feeding can be formed and cause orientation or attraction of fish toward their source, particularly in combination with corresponding visual stimuli and odors.

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