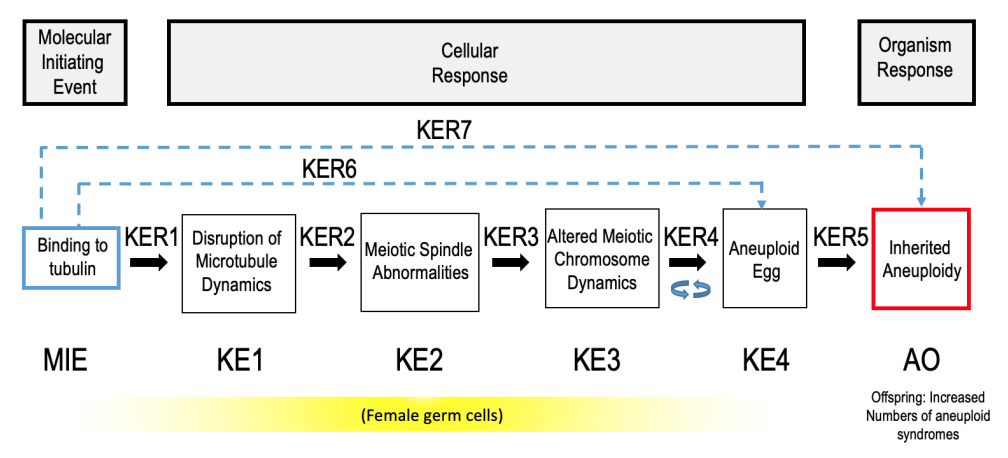
AOP ID and Title:

AOP 106: Chemical binding to tubulin in oocytes leading to aneuploid offspring
Short Title: Tubulin binding and aneuploidy

Graphical Representation



Authors

Francesco Marchetti 1*, Alberto Massarotti 2, Carole L. Yauk 1, Francesca Pacchierotti 3, Antonella Russo 4

1 Environmental Health Science and Research Bureau, Health Canada, Ottawa, Canada. 2 Dipartimento di Scienze del Farmaco, Università degli Studi del Piemonte Orientale “A. Avogadro”, Novara, Italy. 3 Health Protection Technology Division, Laboratory of Biosafety and Risk Assessment, ENEA CR Casaccia, Rome, Italy. 4 Department of Molecular Medicine, University of Padova, Padova, Italy.

- Correspondence to: Francesco Marchetti, Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON K1A 0K9, Canada. E-mail: francesco.marchetti@canada.ca

Status

Author status	OECD status	OECD project	SAAOP status
Open for citation & comment	EAGMST Under Review	1.11	Included in OECD Work Plan

Abstract

Aneuploidy, an abnormal number of chromosomes, arising during meiosis in germ cells represents the most common chromosomal abnormality at birth and is the leading cause of pregnancy loss in humans. Aneuploidy can affect any chromosome, and data in rodents suggest that neither aneuploid sperm nor aneuploid oocytes are selected against at fertilization. Therefore, an increase in germ cell aneuploidy is expected to result in an increase in aneuploid pregnancies. The etiology of human aneuploidy is still not well understood, although there is strong evidence supporting a preferential occurrence during female meiosis I and a positive correlation with maternal age. There is extensive evidence in animal models that chemicals can induce aneuploidy by interfering with the proper functioning of the meiotic spindle and other aspects of chromosome segregation. Over 15 chemicals have been shown to induce aneuploidy in mammalian oocytes and the majority of these chemicals interfere with microtubule dynamics during meiosis. In addition to these animal studies, there is also one reported case in which environmental exposure to trichlorfon, an organophosphate insecticide, was associated with a cluster of Down syndrome cases among women in a Hungarian community. The present AOP focuses on the induction of aneuploidy in mammalian oocytes as a consequence of chemical binding to tubulin (MIE). In this AOP, chemicals that bind to tubulin lead to the depolymerization of microtubules (KE1). Extensive microtubule depolymerization leads to meiotic spindle disorganization (KE2), which in turns lead to altered chromosome dynamics (KE3) and the generation of aneuploid oocytes (KE4). Aneuploid oocytes can be fertilized and generate aneuploid offspring (AO). There is ample empirical evidence supporting this AOP and the overall weight of evidence is strong.

Background

Aneuploidy is associated with serious human health effects. Approximately 10–30% of human zygotes, 50% of spontaneous abortions, and 0.3% of human newborns are aneuploid [Hassold et al., 2007; Nagaoka et al., 2012; Webster and Schuh, 2017]. Cytogenetic analyses of human oocytes and preimplantation embryos have reported frequencies of aneuploidy in excess of 50% [Magli et al., 2001; Munne, 2002; Kuliev et al., 2003]. In these studies, the overall aneuploidy frequency is estimated from the analysis of a subset of chromosomes, which may affect the accuracy of the estimate.

Aneuploidy can affect any chromosome [Nagaoka et al., 2012; Webster and Schuh, 2017], although there is evidence that acrocentric chromosomes may be more frequently involved in aneuploidy than metacentric chromosomes [Nicolaidis and Petersen, 1998; Hassold et al., 2007; Gianaroli et al., 2010]. In humans, only trisomies for a few autosomal chromosomes (13, 18 and 21) and aneuploidies of the sex chromosomes are compatible with life. These aneuploidies have important developmental, neurological and reproductive effects. Trisomy 21 or Down syndrome, with an occurrence of ~1/720 births, is the most common genetic abnormality in newborns [Hassold et al., 2007].

The etiology of human aneuploidy is still not well understood, although there is strong evidencesupporting a preferential occurrence during female meiosis I and a positive correlation with maternal age [Hunt and Hassold, 2002; Nagaoka et al., 2012]. The prevalence of chromosome segregation errors during female meiosis is clearly supported by the application of state-of-the-art genomic approaches, such as Comparative Genomic Hybridization (CGH), array-Comparative Genomic Hybridization (aCGH), SNP-arrays [Handyside, 2012; Nagaoka et al., 2012] and next generation sequencing (NGS) [Hou et al., 2013; Kung et al., 2015; Treff et al., 2016].

The present AOP focuses on chemical binding to tubulin that causes depolymerization of microtubules and generation of aneuploid cells. Although this molecular initiating event can occur in any cell, the adverse outcome is the generation of aneuploid conceptuses; therefore, this AOP is specific to germ cells, and in particular, to female germ cells.

References

Gianaroli L, Magli MC, Cavallini G, Crippa A, Capoti A, Resta S, Robles F, Ferraretti AP. 2010. Predicting aneuploidy in human oocytes: key factors which affect the meiotic process. Hum Reprod 25:2374-2386.

Handyside AH. 2012. Molecular origin of female meiotic aneuploidies. Biochim Biophys Acta 1822:1913-1920.

Hassold T, Hall H, Hunt P. 2007. The origin of human aneuploidy: Where we have been, where we are going. Hum Mol Genet 16: R203–R208.

Hou Y, Fan W, Yan L, Li R, Lian Y, Huang J, Xu L, Tand F, Xiw XS, Qiao J. 2013. Genome analyses of single human oocytes. Cell 155:1492-1506.

Hunt PA, Hassold TJ. 2002. Sex matters in meiosis, Science 296:2181-2183.

Kuliev A, Cieslak J, Ilkevitch Y, Verlinsky Y. 2003. Chromosomal abnormalities in a series of 6,733 human oocytes in preimplantation diagnosis for age-related aneuploidies. Reprod Biomed Online 6:54-59.

Kung A, Munné S, Bankowski B, Coates A, Wells D. 2015. Validation of next-generation sequencing for comprehensive chromosome screening of embryos. Reprod Biomed Online 31:760-769.

Magli MC, Gianaroli L, Ferraretti AP. 2001. Chromosomal abnormalities in embryos. Mol Cell Endocrinol 183:S29-34.

Munne S. 2002. Preimplantation genetic diagnosis of numerical and structural chromosome abnormalities. Reprod Biomed Online 4:183-196.

Nagaoka SI, Hassold TJ, Hunt PA. 2012. Human aneuploidy: Mechanisms and new insights into an age-old problem. Nat Rev Genet 13:493–504.

Nicolaidis P, Petersen MB. 1998. Origin and mechanisms of non-disjunction in human autosomal trisomies. Hum Reprod 13:313-319.

Treff NR, Kirsher RL, Tao X, Garnsey H, Boher C, Silva E, Landis J, Taylor D, Scott RT, Woodruff TK, Duncan FE. 2016. Next Generation Sequencing-based comprehensive chromosome screening in mouse polar bodies, oocytes, and embryos. Biol Reprod 94:76

Webster A, Schuh M. 2017. Mechanisms of aneuploidy in human eggs. Trends Cell Biol 27:55-68.

Summary of the AOP

Events

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

Sequence	Type	Event ID	Title	Short name
1	MIE	718	Binding, Tubulin	Binding, Tubulin
2	KE	720	Disruption, Microtubule dynamics	Disruption, Microtubule dynamics
3	KE	721	Disorganization, Meiotic Spindle	Disorganization, Meiotic Spindle
4	KE	752	Altered, Meiotic chromosome dynamics	Altered, Meiotic chromosome dynamics
5	KE	723	Altered, Chromosome number	Altered, Chromosome number
6	AO	728	Increase, Aneuploid offspring	Increase, Aneuploid offspring

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Binding, Tubulin	adjacent	Disruption, Microtubule dynamics	High	
Disruption, Microtubule dynamics	adjacent	Disorganization, Meiotic Spindle	Moderate	
Disorganization, Meiotic Spindle	adjacent	Altered, Meiotic chromosome dynamics	Moderate	
Altered, Meiotic chromosome				

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
dynamics	adjacent	Altered, Chromosome number	Low	
Altered, Chromosome number	adjacent	Increase, Aneuploid offspring	High	
Binding, Tubulin	non-adjacent	Altered, Chromosome number	High	

Stressors

Name	Evidence
Colchicine	High
Vinblastine sulfate	High
Benomyl	High
Nocodazole	High

Colchicine

This is the prototype agent for binding to tubulin.

Colchicine binds at a site on tublulin known as the colchicine binding domain, which is a deep pocket located at the α/β interface of tubulin heterodimers. Both the A and C rings of colchicine are necessary for high affinity binding, while the B ring may only function as a linker between the other two. Three methoxy residues are present in the A ring and all of them are involved in the high affinity binding to tubulin. The C ring of colchicine interacts through van der Waals contacts with Valα181, Serα178, and Valβ315. The carbonyl group behaves as a hydrogen bond acceptor, interacting with Val181a. The A ring is buried in a hydrophobic pocket delimited by Lysβ352, Asnβ350, Leuβ378, Alaβ316, Leuβ255, Lysβ254, Alaβ250, and Leuβ242, and the methoxy group at position 3 is involved in a hydrogen bond interaction within the thiol group of Cysβ241 [Marchetti et al., 2016].

Vinblastine sulfate

Russo and Pacchierotti (1988) Dose-dependent significant increases of hyperhaploid oocytes at 0.23 mg/kg and 0.45 mg/kg. The percentages of hyperhaploid oocytes were 0.9 (2/219), 0.9 (1/111), 17.2 (26/151), and 59.7 (40/67), for controls, 0.09, 0.23, and 0.45 mg/kg, respectively. At higher doses almost all oocytes are arrested at the metaphase I stage.

Mailhes et al (1993) Significant increases of hyperhaploid oocytes at doses between 0.2 and 0.4 mg/kg. The percentages of hyperhaploid oocytes were 0.5 (1/218), 23.2 (149/641), 23.9 (81/339), 29.3 (72/246) and 30.7 (65/212), for controls, 0.2, 0.25, 0.3 and 0.4 mg/kg, respectively. At 0.6 mg/kg, almost all oocytes are arrested at the metaphase I stage.

Benomyl

Mailhes and Aardema (1992) Hyperhaploidy is significantly increased at all doses tested. The percentages of hyperhaploid oocytes were 0.3 (1/309), 3.9 (6/155), 16.6 (38/229), 35.4 (46/130), 27.9 (60/215) and 29.4 (42/143) for controls, 500, 1000, 1500, 1750 and 2000 mg/kg, respectively. A saturation of the effect is detected for doses above 1500 mg/kg, but its cause is not investigated.

Nocodazole

Sun et al (2005) Hyperhaploidy is significantly increased at 70 mg/kg. The percentages of hyperhaploid oocytes were 0.0 (0/230), 0.0 (0/194) and 7.3 (8/110) for controls, 35 and 70 mg/kg, respectively. The lowest effective dose roughly corresponds to an in vitro concentration of 230 µM, much higher than active concentrations on cultured oocytes. Poor water solubility and limited bioavailability of nocodazole after i.p. treatment likely account for this difference.

Overall Assessment of the AOP

Strong.

A comprehensive review of the literature (Supplementary Table 1) was conducted to gather the available studies in which the effects of microtubule inhibitors were tested for the induction of aneuploidy in female germ cells. The focus of this AOP is on spindle poisons that bind to tubulin resulting in microtubule depolymerization leading to abnormalities in spindle function and chromosomal dynamic ultimately resulting in an egg with an abnormal number of chromosomes. Although chemicals with different mechanism of actions, such as topoisomerase II inhibitors, also have strong data showing aneugenic activity in female germ cells [Mailhes and Marchetti, 2005; Pacchierotti et al., 2007], chemicals that bind to tubulin represent the largest class for which the aneugenic activity has been evaluated [Marchetti et al., 2016]. Studies providing sufficient information regarding doses, timing of exposure and egg collection, and experimental results were considered to assess the empirical data supporting each of the KEs and KERs. It should be noted that, as mentioned before, few studies have investigated multiple KEs within the same study design and the majority of the data available refers to the induction of aneuploidy. An additional complication is that the MIE and first KE (microtubule depolymerization) are most often assessed in acellular systems rendering the quantitative assessment of the concordance among

upstream KEs and downstream KEs complex. However, those few cases where multiple KEs were investigated showed concordance for both dose-related and time-related effects [Shen et al., 2005; Eichenlaub-Ritter et al., 2007]. As a whole, we consider the studies in Supplementary Table 1 to provide extensive and convincing evidence that tubulin-binding chemicals cause microtubule depolymerization and spindle disturbances leading to the generation of aneuploid eggs. Strong in vivo dose-response data on the induction of aneuploid eggs is available for several chemicals, including colchicine, benomyl, and vinblastine [reviewed in Mailhes and Marchetti, 1994; 2005; Pacchierotti et al., 2007]. Data with colchicine is also available to demonstrate that aneuploid eggs are fertilized and that the frequencies of aneuploidy are similar before and after fertilization [Mailhes et al., 1990]. Overall, we consider that the available data provide high support for this AOP as a whole, while empirical support for the different KERs is varied.

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Mus musculus	Mus musculus	High	NCBI
Homo sapiens	Homo sapiens	Moderate	NCBI
Hamster	Hamster	Moderate	NCBI

Sex Applicability

Sex	Evidence
Female	High

Although the molecular initiating event and a few of the key event can occur in any cell type, the adverso outcome is require these events to occur in the oocyte. Thus, the present AOP should be considered specific to female germ cells exposed in the peri-ovulation period. The majority of data in this AOP were derived from experiments in mice, however, relevant endpoints have been evaluated in a variety of higher and lower eukaryotes. The available results on the induction of aneuploidy by the prototype tubulin-binding chemical colchicine in oocytes of species other than *Mus musculus* are qualitatively consistent with mouse data, in agreement with the similarities in the mechanism of action across several Phyla and the high degree of homology of tubulin across species. Evidence for microtubule depolymerization and spindle disorganization has been obtained in human oocytes exposed in culture to colchicine. In addition, the similarities in oogenesis between rodents and humans suggest that the MIE and KEs are conserved and would occur in human oocytes also. Therefore, the AOP should apply to any species that produce eggs.

Essentiality of the Key Events

Not all events within this AOP can be tested for essentiality. This is due to technical limitations at this time. However, there is one study demonstrating the essentiality of proper spindle organization for correct chromosome congression and segregation. Ou et al. [2010] showed that depletion of the microtubule organizing centres (required for spindle organization) leads to increase in the incidence spindle and chromosome dynamic abnormalities. Moreover, studies with mice deficient in specific spindle assembly checkpoint proteins show an increase in the occurrence of high levels of aneuploid oocytes [Leland et al., 2009; McGuinness et al., 2009; reviewed in Mailhes and Marchetti, 2010].

The final step of the AOP requires the transmission of the aneuploid condition from the oocyte to the offspring. Since the available data suggest that there is a narrow window of sensitivity for the induction of aneuploidy by spindle poisons (around the time of resumption of meiosis in preparation for ovulation) it could be possible to wait longer periods of time after the administration of colchicine, or any other of the chemicals listed in this AOP, and demonstrate that under these conditions there is no transmission of aneuploidy to the offspring. However, no such study has been conducted.

Weight of Evidence Summary

Biological plausibility of the KERs is strong. There is clear understanding of the MIE for many of the chemicals listed in this AOP. Both the colchicine- and vinca alkaloid-binding sites on tubulin are characterized in detail. The consequences of chemical binding to tubulin for microtubule dynamics are also qualitatively and quantitatively well understood. It also broadly accepted that microtubule dynamics is essential for proper spindle assembly and function. There is less understanding of why the SAC is unable to prevent meiotic progression in the presence of misaligned chromosomes [Marchetti et al., 2006; Webster and Schuh, 2017] and this represent a key research gap.

Empirical support for the KERs is generally strong, although the empirical evidence and our understanding of the KER between abnormalities in chromosome dynamics and generation of an aneuploidy egg is limited. The strongest empirical support is associated with the indirect KER linking binding of chemicals to tubulin with the induction of aneuploid eggs. Overall, the timescale of events, from the initial biochemical interactions (MIE) occurring within seconds to minutes of exposure, through disruption of

spindle (KE2) and chromosome alignment and segregation in meiosis (KE3) occurring in the following hours, to the formation and ovulation of an aneuploid oocyte (KE4) and to its possible fertilization, which would occur later on, is fully coherent and consistent with the timeline of oocyte development and fertilization [Marchetti et al., 2016]. Moreover, examination of the incidence of events occurring across doses for KE2, KE3 and KE4 after in vitro exposure of oocytes to nocodazole [Shen et al., 2005] and 2-methoxyestradiol [Eichenlaub-Ritter et al., 2007] supports the order and linkages between the KEs across the AOP.

The comparison between the lowest effective concentrations inducing each subsequent event is complex because colchicine binding to tubulin and microtubule depolymerization are measured in acellular systems, whereas, spindle disorganization and altered chromosome alignment and segregation are mostly analysed in cultured oocytes, and induction of aneuploid oocytes and zygotes is assessed after treatment of laboratory rodents by intraperitoneal or oral administrations. Cells in culture may respond to chemical exposure with a different sensitivity than whole organisms [Sun et al., 2005], and a comparison between in vitro molar concentrations and mg/kg body weight of in vivo administered doses can be done only roughly, based on many assumptions. Furthermore, few in vitro experiments were aimed at identifying the Lowest Effective Tested Concentration, or were even conducted at multiple concentration levels. In many cases, experiments aimed to test the hypothesis that a given effect was elicited by chemical disruption of a certain process, and to do this, high doses were used. The published work shows that there is progressivity between dose, severity of spindle damage and degree of aneuploidy, from one to several involved chromosomes up to a complete inhibition of chromosome segregation and arrest of oocytes at meiosis I [Russo and Pacchierotti, 1988; Mailhes et al., 1990; Mailhes and Aardema, 1992; Mailhes et al., 1993; Sun et al., 2005; Eichenlaub-Ritter et al., 2007].

Quantitative Consideration

As described in the previous sections of the AOP, it is well established that chemicals that bind to tubulin affect the polymerization of microtubules triggering abnormalities in the meiotic spindle and the subsequent chromosomal missegregation. There is also sufficient evidence to show that these events increase with dose in a manner that is consistent with this AOP. Binding to tubulin seems to increase linearly with dose, however, microtubule depolymerization must exceed a threshold before abnormalities in the meiotic spindles become apparent. There is also sufficient evidence that there is a threshold for the induction of aneuploidy. However, the precise quantitative relationship has not been established and it may be different for different chemicals. This is because different chemicals may induce different degrees of arrest at the metaphase of the first meiotic division which would prevent the manifestation of the aneuploidy in metaphase II oocytes.

Considerations for Potential Applications of the AOP (optional)

There are no established OECD test guidelines (TG) for measuring aneuploidy in oocytes. However, there are several existing TGs, such as the in vivo and in vitro micronucleus test (OECD TG 474 and OECD TG 487) and the in vivo and in vitro chromosomal aberration test (OECD TG 475 and OECD TG 473), and one specific to spermatogonial cells (OECD TG 483) that although not specifically designed to detect aneuploidy can provide evidence of aneugenic activity. Although it is generally assumed that data obtained in somatic cells can be extrapolated to germ cells to inform regulatory decisions, the availability of germ cell data is critical for the proper classification of products under the Globally Harmonized System (GHS) of classification and labelling [United Nations, 2013]. In addition, the recent International Workshops on Genotoxicity Testing that took place in Tokyo, Japan in November 2017 included a workgroup that addressed the risk of aneugens for human health assessment. As part of the work, the group reviewed all available data for germ cell aneugens in mammals, independently of the mechanism of action. Therefore, the present AOP addresses at topic of high interest among the genotoxicity community and may help in identifying research gaps and direct future work.

References

Eichenlaub-Ritter U, Winterscheidt U, Vogt E, Shen Y, Tinneberg HR, Sorensen R. 2007. 2-methoxyestradiol induces spindle aberrations, chromosome congression failure, and nondisjunction in mouse oocytes. Biol Reprod 76:784–793.

Leland S, Nagarajan P, Polyzos A, Thomas S, Samaan G, Donnell R, Marchetti F, Venkatachalam S. 2009. Heterozygosity for a Bub1 mutation causes female-specific germ cell aneuploidy in mice. Proc Natl Acad Sci USA 106:12776-12781.

Mailhes JB, Marchetti F. 1994. Chemically-induced aneuploidy in mammalian oocytes. Mutat Res 320:87-111.

Mailhes JB, Marchetti F. 2005. Mechanisms and chemically-induced aneuploidy in rodent germ cells. Cytogenet Genome Research 111:384-391.

Mailhes JB, Marchetti F. 2010. Advances in understanding the genetic causes and mechanisms of female germ cell aneuploidy. Exp Rev Obst Gyn 5:687–706.

Mailhes JB, Aardema MJ, Marchetti F. 1993. Investigation of aneuploidy induction in mouse oocytes following exposure to vinblastine-sulfate, pyrimethamine, diethylstilbestrol diphosphate, or chloral hydrate. Environ Mol Mutagen 22:107–114.

Mailhes JB, Carabatsos MJ, Young D, London SN, Bell M, Albertini DF. 1999. Taxol-induced meiotic maturation delay, spindle defects, and aneuploidy in mouse oocytes and zygotes. Mutat Res 423:79-90

Marchetti F, Massarotti A, Yauk CL, Pacchierotti F, Russo A. 2016. The adverse outcome pathway (AOP) for chemical binding to tubulin in oocytes leading to aneuploid offspring. Environ Mol Mutagen 57:87-113.

McGuinness BE, Anger M, Kouznetsova A, Gil-Bernabé AM, Helmhart W, Kudo NR, Wuensche A, Taylor S, Hoog C, Novak B. Nasmyth K. 2009. Regulation of APC/C activity in oocytes by a Bub1-dependent spindle assembly checkpoint. Curr Biol 19:369-380.

OECD. 2016. *Test No. 473: In Vitro Mammalian Chromosomal Aberration Test*, OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264264649-en.

OECD. 2016. *Test No. 474: Mammalian Erythrocyte Micronucleus Test*, OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264264762-en.

OECD. 2016. *Test No. 475: Mammalian Bone Marrow Chromosomal Aberration Test*, OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264264786-en.

OECD. 2016. *Test No. 483: Mammalian Spermatogonial Chromosomal Aberration Test*, OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264264786-en.

OECD. 2016. *Test No. 487: In Vitro Mammalian Cell Micronucleus Test,* OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264264861-en.

Ou XH, Li S, Xu BZ, Wang ZB, Quan S, Li M, Zhang QH, Ouyang YC, Schatten H, Xing FQ, Sun QY. 2010. p38α MAPK is a MTOC-associated protein regulating spindle assembly, spindle length and accurate chromosome segregation during mouse oocyte meiotic maturation. Cell Cycle 9:4130-4143.

Pacchierotti F, Adler ID, Eichenlaub-Ritter U, Mailhes JB. 2007. Gender effects on the incidence of aneuploidy in mammalian germ cells. Environ Res 104:46-69.

Russo A, Pacchierotti F. 1988. Meiotic arrest and aneuploidy induced by vinblastine in mouse oocytes. Mutat Res 202:215–221.

Shen Y, Betzendahl I, Sun F, Tinneberg HR, Eichenlaub-Ritter U. 2005. Non-invasive method to assess genotoxicity of nocodazole interfering with spindle formation in mammalian oocytes. Reprod Toxicol 19:459–471.

Sun F, Betzendahl I, Pacchierotti F, Ranaldi R, Smitz J, Cortvrindt R, Eichenlaub-Ritter U. 2005. Aneuploidy in mouse metaphase II oocytes exposed in vivo and in vitro in preantral follicle culture to nocodazole. Mutagenesis 20:65–75.

United Nations 2013. Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Fifth revised edition ed., New York and Geneva.

Webster A, Schuh M. 2017. Mechanisms of aneuploidy in human eggs. Trends Cell Biol 27:55-68

Appendix 1

List of MIEs in this AOP

Event: 718: Binding, Tubulin

Short Name: Binding, Tubulin

Key Event Component

Process	Object	Action
tubulin binding	tubulin	increased
	tubulin complex	disrupted

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:106 - Chemical binding to tubulin in oocytes leading to aneuploid offspring	MolecularInitiatingEvent

Stressors

Name
Colchicine
Podofilox

Name
518-2-5
Nocodazole
Benomyl
Carbendazim
Thiabendazole
Vincristine

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
eukaryotic cell

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

Colchicine is a prototypical spindle poison that has been extensively used to investigate binding to tubulin. The kinetics of colchicine binding are well established [Lambeir and Engelborghs, 1981; Engelborghs, 1998] and can be measured experimentally with high precision [Hamel and Lin, 1981]. Colchicine binds non-polymerized α/β dimeric tubulin by a two-step process. The first step is rapid but weak, resulting in the formation of an initial pre-equilibrium complex, which involves a low affinity binding of colchicine that is reversible. This is followed by slow conformational changes in tubulin, which finally lead to the formation of an irreversible final state tubulin–colchicine complex that has high activation energy [Garland 1978]. The conformational change in tubulin heterodimers, followed by the addition of the complex at the ends of microtubules, is responsible for the suppressed polymerization at microtubule ends leading to their depolymerization [Ravelli et al., 2004]. The binding kinetics have been studied at different temperatures. The standard enthalpy change of the first step (ΔH°1 = -33±8 kJ · mol–1) and the activation energy of the second step (ΔH°2 = 100±5 kJ · mol–1) were determined based on the temperature dependence [Lambeir and Engelborghs, 1981]. Using eight different analogues to study the binding mechanisms of colchicine, it was demonstrated that the C-ring of colchicine is responsible for the first step of the binding mechanism, while the second step involves the rearrangement of the initial complex to interact with the A-ring [Engelborghs, 1998].

Other chemicals are also known to bind to tubulin [Marchetti et al., 2016]. These chemicals can be grouped in two general classes: colchicine domain binders and vinca domain binders.

Known colchicine domain binders:

1. Podophyllotoxin ((5*R*,5a*R*,8a*R*,9*R*)-5-hydroxy-9-(3,4,5-trimethoxyphenyl)-5a,6,8a,9-tetrahydro-5*H*-[2]benzofuro[5,6-f][1,3]benzodioxol-8-one, POD) has a trimethoxybenzoic chemical structure similar to colchicine. It inhibits colchicine binding to the colchicine-binding domain of tubulin. However, although colchicine and podophyllotoxin bind in the same pocket on β-tubulin, their binding sites are not completely overlapping [Desbene and Giorgi-Renault 2002].

2. 2-methoxyestradiol ((8*R*,9*S*,13*S*,14*S*,17*S*)-2-methoxy-13-methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[a]phenanthrene-3,17-diol, 2ME) binds to the colchicine domain of tubulin [D'Amato et al. 1994].

3. Nocodazole (methyl (5-[2-thienylcarbonyl]-1*H*-benzimidazol-2-yl, NOC) belongs to the group of benzimidazole derivatives that were patented, as a class, for the treatment of cancer in conjunction with other pharmaceuticals. Nocodazole has been shown to bind in the colchicine domain [Xu et al. 2002].

4. Benomyl (methyl *N*-[1-(butylcarbamoyl)benzimidazol-2-yl]carbamate, BEN), another benzimidazole derivative, is the active compound in several agricultural fungicides. The benomyl-binding site is located in the core of β-tubulin at a site distinct from the colchicine domain [Clement et al. 2008].

5. Carbendazim (methyl *N*-(1H-benzimidazol-2-yl)carbamate, MBC) is a fungicide commonly used in agriculture for the control of a wide range of fungal diseases. Carbendazim is the methylbenzimidazolcarbamate product of the spontaneous hydrolyzation process incurred by benomyl in aqueous solution (it is the major metabolite). Therefore, it is at least partially responsible for the benomyl effects observed in vivo. The affinity of carbendazim for mammalian tubulin is less than that of benomyl, most probably because it lacks the position 1 side chain of benomyl. Like benomyl, carbendazim does not compete with colchicine for binding to tubulin [Yenjerla et al., 2008].

6. Thiabendazole (4-(1*H*-benzimidazol-2-yl)-1,3-thiazole, TBZ) is a benzimidazole-derived anthelmintic and an agricultural fungicide, structurally related to NOC, benomyl and MBC. TBZ competitively inhibits MBC binding to fungal tubulin [Davidse and Flach, 1978].

7. ABT-751 (*N*-(2-((4-hydroxyphenyl)amino)pyridin-3-yl)-4-methoxybenzenesulfonamide) has a scaffold based on a benzsulfamide group. It was identified as a potent antiproliferative agent and was subsequently found to be an antitubulin agent by targeting the colchicine binding site [Yoshimatsu et al., 1997].

8. A compound base on m-ethoxyaniline group (2-(6-ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-c]pyrazol-7-yloxy)acetamide) showed noteworthy low nanomolar potency against cancer cell lines. In mechanistic studies, it inhibited tubulin polymerization and disorganized microtubule by binding to tubulin colchicine binding site [Liu et al., 2016].

Vinca domain binders:

The vinca alkaloids, a class of antimitotic compounds derived from the periwinkle plant, *Catharanthus roseus* [Cutts et al., 1960], bind near the GTP-binding site on the β-subunit of tubulin at a site distinct from the colchicine-binding one [Rai and Wolff 1996]. Vinblastine and vincristine are first-generation vinca alkaloids [Kingston, 2009]. At low concentrations, vincas bind to the plus ends of microtubules producing a conformational change of dimers from a straight ‘‘growing’’ vector to a curved ‘‘peeling’’ vector [Toso et al., 1993]. At higher concentrations, the vinca alkaloids have affinity for free tubulin heterodimers, again potentially forming an altered, curved geometry of the dimeric biological vector [Warfield and Bouck, 1974]. Although vincas do not share structural similarity with colchicine and bind to a different site on tubulin, they similarly act by destabilizing microtubules [Stanton et al., 2011].

Vinca domain binders include:

1. Vinblastine (dimethyl (2β,3β,4β,5α,12β,19α)-15-[(5*S*,9*S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4b]indol-9-yl]-3-hydroxy-16-methoxy-1-methyl-6,7-didehydroaspidospermidine-3,4-dicarboxylate, VBL) is an anticancer drug that is used extensively. The crystal structure of vinblastine bound to tubulin has been determined [Gigant et al., 2005]. In contrast with the binding site for colchicine, which is mostly embedded in β-tubulin subunit, the vinblastine binding site is shared equally between α/β-heterodimer [Marchetti et al., 2016]. In the β-subunit, vinblastine interacts through van der Waals contacts with residues Serβ174-Aspβ179, Asnβ206-Aspβ211, Pheβ214 and Tyrβ224; while in the α-subunit, Pheα351, Lysα352, Valα353 and Ileα355 delimit the pocket occupied by VBL. Amino acids Proβ222 and Asnα329 are also involved in hydrogen bond interactions with VBL [Marchetti et al., 2016]. Following a mechanism similar to colchicine, VBL binds to tubulin in two consecutive steps: formation of a rapid equilibrium complex followed by a slower rearrangement linked to changes in the structure of the heterodimer. A major effect of VBL is the formation of spiral-like tubulin aggregates [Weisenberg and Timasheff, 1970; Himes, 1991]. VBL binds to microtubule ends [Wilson et al., 1982] and at low concentrations suppresses the dynamic instability of plus ends [Toso et al., 1993]. When used at much higher concentrations, VBL depolymerizes microtubules, giving rise in particular to protofilament spirals and curls.

2. Vincristine (methyl (3a*R*,3a^1*R*,4*R*,5*S*,5a*R*,10b*R*)-4-acetoxy-3a-ethyl-9-((5*S*,7*S*,9*S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methano[1]azacycloundecino[5,4-*b*]indol-9-yl)-6-formyl-5-hydroxy-8-methoxy-3a,3a^1,4,5,5a,6,11,12-octahydro-1*H*-indolizino[8,1-cd]carbasole-5-carboxylate, VCR) is an anticancer drug that binds to tubulin in the vinca binding domain [Kingston, 2009].

3. Vinflunine (methyl (3a*R*,3a1*R*,4*R*,5*S*,5a*R*,10b*R*)-4-acetoxy-9-((4*R*,6*R*,8*S*)-4-(1,1-difluoroethyl)-8-(methoxycarbonyl)-1,3,4,5,6,7,8,9-octahydro-2,6-methanoazecino[4,3-b]indol-8-yl)-3a-ethyl-5-hydroxy-8-methoxy-6-methyl-3a,3a1,4,5,5a,6,11,12-octahydro-1*H*-indolizino[8,1-cd]carbazole-5-carboxylate) is a florinated Vinca alkaloid, like vinblastine and vincristine, and appears to interact at the Vinca binding domain [Kruczynski et al., 1998].

4. Vintafolide ((2*R*,5*S*,8*S*,11*S*,14*S*,19*S*)-19-(4-(((2-amino-4-oxo-3,4-dihydropteridin-6-yl)methyl)amino)benzamido)-5,8,14-tris(carboxymethyl)-2-(((2-((2-((3a*R*,3a1*R*,4*R*,5*S*,5a*R*,10b*R*)-3a-ethyl-9-((5*S*,7*R*,9*S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methano[1]azacycloundecino[5,4-b]indol-9-yl)-4,5-dihydroxy-8-methoxy-6-methyl-3a,3a1,4,5,5a,6,11,12-octahydro-1*H*-indolizino[8,1-cd]carbazole-5-carbonyl)hydrazine-1-carbonyl)oxy)ethyl)disulfanyl)methyl)-11-(3-guanidinopropyl)-4,7,10,13,16-pentaoxo-3,6,9,12,15-pentaazaicosanedioic acid) is a drug conjugate consisting of a small molecule targeting the folate receptor and vinblastine. Vintafolide is designed to deliver vinblastine selectively to cells over-expressing the folate receptor such as ovarian cancer cells [Vergote and Leamon, 2015].

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
Homo sapiens	Homo sapiens	Moderate	NCBI
rat	Rattus norvegicus	Moderate	NCBI
Xenopus laevis	Xenopus laevis	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Chemical binding to tubulin has been measured in somatic and germ cells in a variety of species, from rodents in vivo to human cells in culture. Theoretically, chemical binding to tubulin can occur in any cell type in any organism.

Key Event Description

The site of action is the tubulin in the cytoplasm. Tubulins represent a large superfamily, and several isotypes are described for both α and β tubulin in mammalian cells [Luduena, 2013]. At least six different isotypes of the α subunit are known, while eight isotypes are known for the β subunit. These subunits share a high degree of homology (90% similarity). In addition to α- and β-tubulin, other tubulin homologues have been identified (γ, δ and ε), but their roles in the life cycle of the cell are uncertain [Bhattacharya and Cabral, 2009]. All available isotypes are incorporated within microtubules, although with different tissue distributions in normal cells [Berrieman et al., 2004]. The currently known microtubule-disrupting agents bind to all isotypes, having only a slight preference for one over another [Miller et al., 2010].

Binding sites on the α/β-tubulin heterodimer: Conventionally, microtubule-interfering agents are categorized into two main groups: (1) microtubule destabilizers, including colchicine and a variety of vinca alkaloids; and (2) microtubule stabilizers, including taxanes and epothilones. Most agents interact with known binding pockets of α/β-tubulin; however, there are compounds that bind to tubulin on undefined sites. Three distinct sites are well characterized in the literature [Marchetti et al., 2016; Botta et al., 2009]: (1) the colchicine-binding domain at the interface between the α- and β-tubulin dimers; (2) the vinca domain surrounding the GTP binding site on β- and α-tubulin; and, (3) the taxane domain located on β-tubulin [Botta et al., 2009].

Colchicine binding domain on tubulin: The colchicine binding domain is a deep pocket located at the α/β interface of tubulin heterodimers. Crystal structures for tubulin and different ligands are available, although their resolution is not high [Lu et al., 2012; Massarotti et al., 2012]. Notwithstanding its deep location, significant conformational changes in the protein are necessary for accommodating the inhibitors. Both the A and C rings of colchicine are necessary for high affinity binding, while the B ring may only function as a linker between the other two. Three methoxy residues are present in the A ring and all of them are involved in the high affinity binding to tubulin. The C ring of colchicine interacts through van der Waals contacts with Valα181, Serα178, and Valβ315. The carbonyl group behaves as a hydrogen bond acceptor, interacting with Val181a. The A ring is buried in a hydrophobic pocket delimited by Lysβ352, Asnβ350, Leuβ378, Alaβ316, Leuβ255, Lysβ254, Alaβ250, and Leuβ242, and the methoxy group at position 3 is involved in a hydrogen bond interaction within the thiol group of Cysβ241 [Marchetti et al., 2016]. Different ligands may compete with colchicine for the same binding site, even in the absence of high structural correspondence [Lu et al., 2012].

There is no OECD guideline for measuring chemical binding to tubulin, however, binding of colchicine to tubulin is one of the most studied chemical interactions with biological materials and methodology for its measurement is well established and standardized [Hamel and Lin, 1981; Verdier-Pinard et al., 1998].

How it is Measured or Detected

Binding properties to tubulin are generally evaluated in vitro, typically on tubulin extracts derived from brain tissues [Miller and Wilson 2010]. To determine whether a compound can bind to tubulin, a competitive [3H]colchicine tubulin-binding assay is conducted in vitro to measure whether the binding of colchicine is inhibited by the presence of the test agent [Verdier-Pinard et al. 1998]. A reaction mixture containing tubulin, [3H]colchicine and a potential inhibitor is incubated and after the addition of the scintillation fluid, the radioactivity of [3H]colchicine-bound tubulin is measured using a scintillation counter. The reduction of [3H]colchicine-bound tubulin value is inversely proportional to the test agent binding affinity [Hamel and Lin 1981]. A reaction mixture with only tubulin and [3H]colchicine is generally used as an experimental control standard. The inhibition constant (Ki) of colchicine is 5.75 µM [Zavala et al. 1980] and the ability of new chemicals to interfere with colchicine binding to tubulin is benchmarked against this value.

References

Berrieman HK, Lind MJ, Cawkwell L. 2004. Do beta-tubulin mutations have a role in resistance to chemotherapy? Lancet Oncol 5:158-164.

Bhattacharya R, Cabral F. 2009. Molecular basis for class V beta-tubulin effects on microtubule assembly and paclitaxel resistance. J Biol Chem 284:13023-13032.

Botta M, Forli S, Magnani M, Manetti F. 2009. Molecular Modeling Approaches to Study the Binding Mode on Tubulin of Microtubule Destabilizing and Stabilizing Agents. In: Carlomagno T, editor. Tubulin-Binding Agents: Springer Berlin Heidelberg. p 279-328.

Clement MJ, Rathinasamy K, Adjadj E, Toma F, Curmi PA, Panda D. 2008. Benomyl and colchicine synergistically inhibit cell proliferation and mitosis: evidence of distinct binding sites for these agents in tubulin. Biochemistry 47:13016-13025.

Cutts JH, Beer CT, Noble RL. 1960. Biological properties of Vincaleukoblastine, an alkaloid in Vinca rosea Linn, with reference to its antitumor action. Cancer Res 20:1023-1031.

D'Amato RJ, Lin CM, Flynn E, Folkman J, Hamel E. 1994. 2-Methoxyestradiol, an endogenous mammalian metabolite, inhibits tubulin polymerization by interacting at the colchicine site. Proc Natl Acad Sci USA 91:3964-3968.

Desbene S, Giorgi-Renault S. 2002. Drugs that inhibit tubulin polymerization: the particular case of podophyllotoxin and analogues. Curr Med Chem Anticancer Agents 2:71-90.

Engelborghs Y. 1998. General features of the recognition by tubulin of colchicine and related compounds. Eur Biophys J 27:437-445.

Garland DL. 1978. Kinetics and mechanism of colchicine binding to tubulin: evidence for ligand-induced conformational change. Biochemistry 17:4266-4272.

Gigant B, Wang C, Ravelli RB, Roussi F, Steinmetz MO, Curmi PA, Sobel A, Knossow M. 2005. Structural basis for the regulation of tubulin by vinblastine. Nature 435:519-522.

Hamel E, Lin CM. 1981. Stabilization of the colchicine-binding activity of tubulin by organic acids. Biochim Biophys Acta 675:226-231.

Himes RH. 1991. Interactions of the catharanthus (Vinca) alkaloids with tubulin and microtubules. Pharmacol Ther 51:257-267.

Kingston DG. 2009. Tubulin-interactive natural products as anticancer agents. J Nat Prod 72:507-515.

Lambeir A, Engelborghs Y. 1981. A fluorescence stopped flow study of colchicine binding to tubulin. J Biol Chem 256:3279-3282.

Lu Y, Chen J, Xiao M, Li W, Miller DD. 2012. An overview of tubulin inhibitors that interact with the colchicine binding site. Pharm Res 29:2943-2971.

Luduena RF. 2013. A hypothesis on the origin and evolution of tubulin. Int Rev Cell Mol Biol 302:41-185.

Marchetti A, Massarotti A, Yauk CL, Pacchierotti F, Russo A. Submitted. The adverse outcome pathway (AOP) for chemical binding to tubulin in oocytes leading to aneuploid offspring. Environ Mol Mutagen.

Massarotti A, Coluccia A, Silvestri R, Sorba G, Brancale A. 2012. The tubulin colchicine domain: a molecular modeling perspective. ChemMedChem 7:33-42.

Miller HP, Wilson L. 2010. Chapter 1 - Preparation of Microtubule Protein and Purified Tubulin from Bovine Brain by Cycles of Assembly and Disassembly and Phosphocellulose Chromatography. In: Leslie W, John JC, editors. Methods Cell Biol: Academic Press. p 2-15.

Miller LM, Xiao H, Burd B, Horwitz SB, Angeletti RH, Verdier-Pinard P. 2010. Chapter 7 - Methods in Tubulin Proteomics. In: Leslie W, John JC, editors. Methods Cell Biol: Academic Press. p 105-126.

Rai SS, Wolff J. 1996. Localization of the vinblastine-binding site on beta-tubulin. J Biol Chem 271:14707-14711.

Ravelli RB, Gigant B, Curmi PA, Jourdain I, Lachkar S, Sobel A, Knossow M. 2004. Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. Nature 428:198-202.

Stanton RA, Gernert KM, Nettles JH, Aneja R. 2011. Drugs that target dynamic microtubules: a new molecular perspective. Med Res Rev 31:443-481.

Toso RJ, Jordan MA, Farrell KW, Matsumoto B, Wilson L. 1993. Kinetic stabilization of microtubule dynamic instability in vitro by vinblastine. Biochemistry 32:1285-1293.

Warfield RK, Bouck GB. 1974. Microtubule-macrotubule transitions: intermediates after exposure to the mitotic inhibitor vinblastine. Science 186:1219-1221.

Weisenberg RC, Timasheff SN. 1970. Aggregation of microtubule subunit protein. Effects of divalent cations, colchicine and vinblastine. Biochemistry 9:4110-4116.

Wilson L, Jordan MA, Morse A, Margolis RL. 1982. Interaction of vinblastine with steady-state microtubules in vitro. J Mol Biol 159:125-149.

Xu K, Schwarz PM, Ludueña RF. 2002. Interaction of nocodazole with tubulin isotypes. Drug Dev Res 55:91-96.

Zavala F, Guenard D, Robin JP, Brown E. 1980. Structure--antitubulin activity relationship in steganacin congeners and analogues.

Inhibition of tubulin polymerization in vitro by (+/-)-isodeoxypodophyllotoxin. J Med Chem 23:546-549.

List of Key Events in the AOP

Event: 720: Disruption, Microtubule dynamics

Short Name: Disruption, Microtubule dynamics

Key Event Component

Process	Object	Action
microtubule depolymerization	microtubule	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:106 - Chemical binding to tubulin in oocytes leading to aneuploid offspring	KeyEvent

Stressors

Name
Colchicine
colchemid
Vinblastine sulfate

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
eukaryotic cell

Evidence for Perturbation by Stressor

Colchicine

Colchicine interferes with microtubule dynamics at lower concentrations while it induces a net depolymerization at higher concentrations which is a consequence of the inability of further extending the microtubules [Stanton et al., 2011]. This dual action is in common with other spindle poisons (e.g. vinca derivatives) [Panda et al., 1996]. All microtubule-binding agents alter microtubule dynamics, engaging cell cycle surveillance mechanisms that arrest cell division in metaphase. This mitotic stall may then lead to various irremediable effects such as mitotic catastrophe, apoptosis or aneuploidy [Kops et al., 2005].

After addition of colchicine at concentrations of 0.1-3.0 mM, microtubule polymerization decreased rapidly and simultaneously thoughout the central spindle and aster (Salmon et al, 1984)

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
Homo sapiens	Homo sapiens	Moderate	NCBI
Xenopus laevis	Xenopus laevis	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Depolymerization of microtubules has been measured in many somatic cell types, in addition to frog and mouse eggs, and in human cells, including eggs, in culture [Salmon et al. 1984; Wilson et al., 1984; Ibanez et al., 2003; Liu et al., 2010]. Quantitative cell-based assays for assessing microtubule activities of compounds are achieved by measuring the indirect effects on cell cycle which result from a disruption of microtubule networks. These methods utilize either fluorescent microscopy or cell cycle analysis. In fluorescent microscopic studies, either the α- or β-tubulin can be labeled directly with a tubulin antibody-conjugated fluorescent probe, or indirectly via a secondary antibody [Zhou et al., 2009]. Tubulin stabilizers and destabilizers cause cell cycle arrest at the G2/M phase [Bhalla, 2003], and therefore measurement of the percentage of cells arrested in G2/M phase is used as a surrogate endpoint for microtubule activity.

Key Event Description

Microtubules are polar structures, and in each filament, subunits are added to one extremity (the plus end) and removed from the other one (the minus end) [reviewed in Marchetti et al. 2016]. Microtubules are dynamic structures characterized by features such as dynamic instability and treadmilling. Dynamic instability defines the ability of microtubules to grow or shorten [Mitchison & Kirschner, 1984; Wade and Hyman, 1997]; the process is based on a multitude of events regulating the assembly/disassembly of the subunits. Treadmilling is the process by which, in the presence of an active loss of subunits (at the minus end) and acquisition of subunits (at the plus end), a steady-state is maintained, and the length of the microtubule remains unchanged [Waterman-Sloter and Salmon, 1997]. Microtubule dynamics can be affected as a result of microtubule depolymerization or microtubule stabilization.

How it is Measured or Detected

Microtubule depolymerization is generally assessed by an acellular tubulin polymerization assay [Salmon et al., 1984; Wilson et al., 1984; Wallin and Hartley-Asp, 1993; Ibanez et al., 2003; Liu et al., 2010]. A reaction mixture containing tubulin and a test agent, after preincubation, is chilled on ice. GTP is added, and turbidity development is followed at 350 nm in a temperature-controlled recording spectrophotometer. The extent of the reaction is then measured and the area under the curve is used to determine the concentration that inhibited tubulin polymerization by 50% (IC50) [Hamel, 2003]. A concentration of 2.5 µM of colchicine is needed to inhibit microtubule polymerization by 50% [Zavala et al., 1980] and the ability of new chemicals to induce this effect is benchmarked against this value (e.g., combretastatin A-4 IC50 is 1.2 µM [Pettit et al., 1998]).

References

Bhalla KN. 2003. Microtubule-targeted anticancer agents and apoptosis. Oncogene 22:9075-9086.

Hamel E. 2003. Evaluation of antimitotic agents by quantitative comparisons of their effects on the polymerization of purified tubulin. Cell Biochem Biophys 38:1-22.

Ibanez E, Albertini DF, Overstrom EW. 2003. Demecolcine-induced oocyte enucleation for somatic cell cloning: coordination between cell-cycle egress, kinetics of cortical cytoskeletal interactions, and second polar body extrusion. Biol Reprod 68:1249-1258.

Kops GJ, Weaver BA, Cleveland DW. 2005. On the road to cancer: aneuploidy and the mitotic checkpoint. Nat Rev Cancer 5:773-785.

Lambrus BG, Holland AJ. 2017. A new mode of mitotic surveillance. Trends Cell Biol 27:314-321.

Liu S, Li Y, Feng HL, Yan JH, Li M, Ma SY, Chen ZJ. 2010. Dynamic modulation of cytoskeleton during in vitro maturation in human oocytes. Am J Obstet Gynecol 203:151.e151-157.

Mailhes JB, Carabatsos MJ, Young D, London SN, Bell M, Albertini DF. 1999. Taxol-induced meiotic maturation delay, spindle defects, and aneuploidy in mouse oocytes and zygotes. Mutat Res 423:79-90.

Marchetti F, Massarotti A, Yauk CL, Pacchierotti F, Russo A. 2016. The adverse outcome pathway (AOP) for chemical binding to tubulin in oocytes leading to aneuploid offspring. Environ Mol Mutagen 57:87-113.

Mitchison T, Kirschner M. 1984. Dynamic instability of microtubule growth. Nature. 312:237-242.

Panda D, Jordan MA, Chu KC, Wilson L. 1996. Differential effects of vinblastine on polymerization and dynamics at opposite microtubule ends. J Biol Chem 271:29807-29812.

Pettit GR, Toki B, Herald DL, Verdier-Pinard P, Boyd MR, Hamel E, Pettit RK. 1998. Antineoplastic agents. 379. Synthesis of phenstatin phosphate. J Med Chem 41:1688-1695.

Salmon ED, McKeel M, Hays T. 1984. Rapid rate of tubulin dissociation from microtubules in the mitotic spindle in vivo measured by blocking polymerization with colchicine. J Cell Biol 99:1066-1075.

Stanton RA, Gernert KM, Nettles JH, Aneja R. 2011. Drugs that target dynamic microtubules: a new molecular perspective. Med Res Rev 31:443-481.

Wade RH, Hyman AA. 1997. **Microtubule structure and dynamics.** Curr Opin Cell Biol 9:12-17.

Wallin M, Hartley-Asp B. 1993. Effects of potential aneuploidy inducing agents on microtubule assembly in vitro. Mutat Res 287:17-22.

Waterman-Sloter CM, Salmon ED. 1997. Microtubule dynamics: treadmilling comes around again. Curr Biol 7:R369-R372.

Wilson L, Miller HP, Pfeffer TA, Sullivan KF, Detrich HW, 3rd. 1984. Colchicine-binding activity distinguishes sea urchin egg and outer doublet tubulins. J Cell Biol 99:37-41.

Zavala F, Guenard D, Robin JP, Brown E. 1980. Structure--antitubulin activity relationship in steganacin congeners and analogues. Inhibition of tubulin polymerization in vitro by (+/-)-isodeoxypodophyllotoxin. J Med Chem 23:546-549.

Zhou YB, Feng X, Wang LN, Du JQ, Zhou YY, Yu HP, Zang Y, Li YJ, Li J. 2009. LGH00031, a novel ortho-quinonoid inhibitor of cell division cycle 25B, inhibits human cancer cells via ROS generation. Acta Pharmacol Sin 30;1359-1368.

Event: 721: Disorganization, Meiotic Spindle

Short Name: Disorganization, Meiotic Spindle

Key Event Component

Process	Object	Action
spindle organization	spindle	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:106 - Chemical binding to tubulin in oocytes leading to aneuploid offspring	KeyEvent

Stressors

Name
Colchicine
Nocodazole
Colcemid

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term

eukaryotic cell
Cell term

Evidence for Perturbation by Stressor

Colchicine

In vitro treatment with 0.4 micrograms/mL (1 microM) induces reduction of spindle size and lowe microtubule density; cytoskeleton remodeling is also observed (Ibanez et al 2003). In addtion, colchicine treament results in abnormal spindle localization of several proteins that are essential for chormosome segregation, such as: Aurora A (Yao et al 2004); Polo-like-kinase I (Yao et al 2003; Tong et al 2002); GTPase Ran (Cao et al, 2005)

Nocodazole

In vitro expsoure of oocytes to 20 microgram/mL (67 microM) Nocodazole causes a gradual disassembly of the spindle , which is completed within 15 minutes (Xu et al 2002)

Colcemid

After in vitro treatment wiht 0.4 microgram/mL (1 microgramM) reduction of spindle size and lower microtubule density is detected in activated oocytes with respect to controls; cytoskeleton remodelling is also observed (Ibanez et al 2003).

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

All eukaryotic cells possess a spindle that must be properly organized for normal cellular division. Thus, this key event, although typically measured in mouse and human cells, is theoretically relevant to any eukaryotic cell type.

Key Event Description

The spindle is a cytoskeletal structure present in every eukaryotic cell that must form before cell division in order to properly separate chromosomes between daughter cells [Prosser and Pelletier, 2017]. The spindle organizes itself in a bipolar configuration within the cell prior to cell division. Several hundred proteins are required to assemble a functioning spindle, and microtubules are the most abundant components of the machinery. Although the function of the spindle is similar between mitotic and meiotic cells, spindle formation occurs via distinct mechanisms in female germ cells with respect to other cell types (including male germ cells) [Dumont and Desai, 2012]. This is because spindle formation is generally driven by centrioles, which are lacking in eggs [Szollosi et al., 1972; Manandhar et al., 2005]. The processes in somatic cells and male germ cells versus those operating in oocytes are briefly described below. In this key event, a bipolar spindle configuration is not achieved. Alternatively, there may be some spindle fibers that are not of the appropriate length, shape or structure to ensure that chromosomes can be properly aligned at metaphase and equally distributed between daughter cells.

Somatic cells and male germ cells:

The spindle of mitotic cells and that of male germ cells is organized by the centrosome which is composed by a pair of centrioles surrounded by an amorphous pericentriolar material containing more than 100 proteins [Andersen et al., 2003]. Many proteins that are involved in regulating microtubule dynamics and spindle assembly checkpoint (SAC) are contained in the centrosome. The centrosome is the principal microtubule-organizing center (MTOC) in mammalian cells and plays a major role in controlling microtubule dynamics, nucleation, and kinetochore–microtubule attachments [Conduit et al., 2015]. Errors in these processes lead to structural and functional abnormalities in the mitotic spindle [Rivera-Rivera and Saavedra, 2016].

Centriole and centrosome duplication are tightly coordinated with DNA replication, mitosis, and cytokinesis and play key roles in regulating transitions through the cell cycle [Chan, 2011]. The centrioles, cylindrical particles composed by nine triplet microtubules [Gogendeau et al., 2015], duplicate by forming daughter centrioles oriented at right angles with respect to the parent centrioles and then become surrounded by separate pericentriolar material during S-phase [Bettencourt-Dias and Glover, 2007]. Before mitosis, the newly formed centrosomes move to the opposite site of the nucleus and originate the two poles of the mitotic spindle [Kellog, 1989; Paintrand et a.l, 1992; Chavali et al., 2012]. Microtubules begin to radiate away from the centrosome and move toward the metaphase plate forming the mitotic spindle. During the assembly of the mitotic spindle, some microtubule fibers attach to the kinetochores on chromosomes, some radiate from the spindle poles toward the cell cortex and others extend past the metaphase plate forming a region of overlap with spindle fibers originating from the opposite centrosome [Cassimeris and Skibbens, 2003; Prosser and Pelletier, 2017]. Although a bipolar spindle can be formed in the absence of centrosomes, having too many centrosomes can result in a morphologically abnormal spindle and increase the chance of chromosome missegration [Hinchcliffe, 2014; Nigg and Holland, 2018].

Oocytes:
In mammalian oocytes, centrioles and centrosomes are absent [Manandhar et al.. 2005] and the meiotic spindle starts its growth from several MTOCs that substitute for the conventional centrosome pair. A mouse oocyte can have up to 80 of these MTOCs [Dumont and Desai, 2012]. These MTOCs gradually coalesce and surround the chromosomes [Schuh and Ellenberg, 2007]. Then, microtubules elongate forming a barrel-shape bipolar spindle. Recent data suggest that MTOCs undergo a three-step decondensation and fragmentation process that facilitate their equal distribution to the spindle poles [Clift and Schuh, 2015]. In addition, recent evidence has shown the presence of actin fibers in the mammalian oocyte spindle that are important for ensuring proper chromosome segregation [Mogessie and Schuh, 2017]. Evidence is also emerging about differences in spindle assembly between rodent and human oocytes. Specifically, human oocytes may lack MTOCs and spindle assembly is mediated by chromosomes and the small guanosine triphosphate Ran [Holubcová et al., 2015].

How it is Measured or Detected

Spindle abnormalities in its structure and shape that can be recorded are: reduction of microtubule density, loss of barrel shape, monopolar or multipolar spindle, reduced distance between the poles [Ibanez et al., 2003; Shen et al., 2005; Eichenlaub-Ritter et al., 2007; Xu et al., 2012]. In addition, the use of enhanced polarizing microscope (Polscope/SpindleViewTM) allows the detection of reduction in the birefringency and reduced light retardance of the spindle, which are indicators of loss of organization, at doses below which spindle abnormalities are detected with more conventional immunofluorescence methods [Shen et al., 2005].

Spindle organization is generally assessed by fluorescent immunodetection of its components and confocal microscopy [Ibanez et al., 2003; Shen et al., 2005; Eichenlaub-Ritter et al., 2007; Xu et al., 2012]. Localization of proteins with a known role in spindle function is also assessed [Tong et al., 2002; Yao et al., 2004; Cao et al., 2005]. 3D live imaging of cells expressing fluorescent-tagged proteins provides the possibility to follow spindle function at high resolution, and to describe and measure abnormal parameters (e.g., spindle morphology, altered distance between the two poles, mono- or multipolarity) [Schuh and Ellenberg, 2007]. Enhanced polarizing microscope has also been used to assess spindle integrity in human oocytes during in vitro fertilization techniques [Wang et al., 2001a,b; Keefe et al., 2003; Staessen et al., 1997].

References

Andersen JS, Wilkinson CJ, Mayor T, Mortensen P, Nigg EA, Mann M. 2003. Proteomic characterization of the human centrosome by protein correlation profiling. Nature 426:570-574.

Bettencourt-Dias M, Glover DM. 2007. Centrosome biogenesis and function: centrosomics brings new understanding. Nat Rev Mol Cell Biol 8:451-463.

Cao Y-K, Zhong Z-S, Chen D-Y, Zhang G-X, Schatten H, Sun Q-Y. 2005. Cell cycle-dependent localization and possible roles of the small GTPase Ran in mouse oocyte maturation, fertilization and early cleavage. Reproduction 130:431-440.

Cassimeris L, Skibbens RV. 2003. Regulated assembly of the mitotic spindle: a perspective from two ends. Curr Issues Mol Biol 5:99-112.

Chan JY. 2011. A clinical overview of centrosome amplification in human cancers. Int J Biol Sci 7:1122-1144.

Chavali PL, Peset I, Gergely F. 2012. Centrosomes and mitotic poles: a recent liason? Biochem Soc Trans 43:13-18.

Conduit PT, Wainman A, Raff JW. 2015. Centrosome function and assembly in animal cells. Nat Rev Mol Cell Biol 16:611-624.

Clift D, Schuh M. 2015. A three-step MTOC fragmentation mechanism facilitate bipolar spindle assembly in mouse oocytes. Nat Commun 6:7217, 10.1038/ncomm8217.

Dumont J, Desai A. 2012. Acentrosomal spindle assembly and chromosome segregation during oocyte meiosis. Trends Cell Biol 22: 241-249.

Eichenlaub-Ritter U, Winterscheidt U, Vogt E, Shen Y, Tinneberg HR, Sorensen R. 2007. 2-methoxyestradiol induces spindle aberrations, chromosome congression failure, and nondisjunction in mouse oocytes. Biol Reprod 76:784-793.

Gogendeau D, Guichard P, Tassin AM. 2015. Purification of centrosomes from mammalian cell lines. Methods Cell Biol. 129:171-189.

Hinchcliffe EH. 2014. Centrosomes and the art of mitotic spindle maintenance. Int Rev Cell Mol Biol 313:179-217.

Holubcová Z, Blayney M, Elder K, Schuh M. 2015. Error-prone chromosome-mediated spindle assembly favors chromosome segregation defects in human oocytes. Science 348:1143-1147.

Ibanez E, Albertini DF, Overstrom EW. 2003. Demecolcine-induced oocyte enucleation for somatic cell cloning: coordination between cell-cycle egress, kinetics of cortical cytoskeletal interactions, and second polar body extrusion. Biol Reprod 68:1249-1258.

Keefe D, Liu L, Wang W, Silva C. 2003. Imaging meiotic spindles by polarization light microscopy: principles and applications to IVF. Reprod Biomed Online 7:24–29.

Kellog DR. 1989. Centrosomes. Organizing cytoplasmic events. Nature 340:99-100.

Manandhar G, Schatten H, Sutovsky P. 2005. Centrosome reduction during gametogenesis and its significance. Biol Reprod 72:2-13.

Marchetti F, Massarotti A, Yauk CL, Pacchierotti F, Russo A. 2016. The adverse outcome pathway (AOP) for chemical binding to tubulin in oocytes leading to aneuploid offspring. Environ Mol Mutagen 57:87-113.

Mogessie B, Schuh M. 2017. Actin protects mammalian eggs against chromosome segregation errors. Science Aug 25;357(6353). pii: eaal1647.

Nigg EA, Holland AI. 2018. Once and only once: mechanisms of centriole duplication and their deregulation in disease. Nat Rev Mol Cell BIol 19:297-312.

Paintrand M, Moudjou M, Delacroix H, Bornens M. 1992. Centrosome organization and centriole architecture: their sensitivity to divalent cations. *J Struct Biol* 108:107–128.

Prosser SL, Pelletier L. 2017. Mitotic spindle assembly in animal cells: a fine balancing act. Nat Rev Mol Cell Biol 18:187-201.

Rivera-Rivera Y, Saavedra HI. Centrosome - a promising anti-cancer target. 2016. Biologics 10:167-176.

Schuh M, Ellenberg J. 2007. Self-organization of MTOCs replaces centrosome function during acentrosomal spindle assembly in live mouse oocytes. Cell 130:484-498.

Shen Y, Betzendahl I, Sun F, Tinneberg HR, Eichenlaub-Ritter U. 2005. Non-invasive method to assess genotoxicity of nocodazole interfering with spindle formation in mammalian oocytes. Reprod Toxicol 19:459-471.

Staessen C, Van Steirteghem AC. 1997. The chromosomal constitution of embryos developing from abnormally fertilized oocytes after intracytoplasmic sperm injection and and conventional in-vitro fertilization. Hum Reprod 12:321–327.

Szollosi D, Calarco P, Donahue RP. 1972. Absence of centrioles in the first and second meiotic spindles of mouse oocytes. J Cell Sci 11:521-541.

Tong C, Fan H-Y, Lian L, Li S-W, Chen D-Y, Schatten H, Sun Q-Y. 2002. Polo-like kinase-1 is a pivotal regulator of microtubule assembly during mouse oocyte meiotic maturation, fertilization, and early embryonic mitosis. Biol Reprod 67:546-554.

Wang WH, Meng L, Hackett RJ, Odenbourg R, Keefe DL. 2001a. The spindle observation and its relationship with fertilization after intracytoplasmic sperm injection in living human oocytes. Fertil Steril 75:348–353.

Wang WH, Meng L, Hackett RJ, Odenbourg R, Keefe DL. 2001b. Limited recovery of meiotic spindles in living human oocytes after cooling–rewarming observed using polarized light microscopy. Hum Reprod 16:2374–2378.

Xu XL, Ma W, Zhu YB, Wang C, Wang BY, An N, An L, Liu Y, Wu ZH, Tian JH. 2012. The microtubule-associated protein ASPM regulates spindle assembly and meiotic progression in mouse oocytes. PLoS One 7:e49303.

Yao LJ, Fan HY, Tong C, Chen DY, Schatten H, Sun QY. 2003. Polo-like kinase-1 in porcine oocyte meiotic maturation, fertilization and early embryonic mitosis. Cell Mol Biol 49:399-405.

Yao L-J, Zhong Z-S, Zhang L-S, Chen D-Y, Schatten H, Sun Q-Y. 2004. Aurora-A is a critical regulator of microtubule assembly and nuclear activity in mouse oocytes, fertilized eggs, and early embryos. Biol Reprod 70:1392-1399.

Event: 752: Altered, Meiotic chromosome dynamics

Short Name: Altered, Meiotic chromosome dynamics

Key Event Component

Process	Object	Action
chromosome movement towards spindle pole	chromosome	abnormal

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:106 - Chemical binding to tubulin in oocytes leading to aneuploid offspring	KeyEvent
Aop:396 - Deposition of ionizing energy leads to population decline via impaired meiosis	KeyEvent

Stressors

Name
2-Methoxyestradiol
Nocodazole

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
eukaryotic cell

Evidence for Perturbation by Stressor

2-Methoxyestradiol

After in vitro exposure, bipolar spindle formation and chromosome alignment at the metaphase plate are severely disturbed in oocytes. Six hr later, the percentage of aneuploid oocytes is significantly increased about the control level (Eichenlaub-RItter et al 2007).

Nocodazole

All tested concentrations induce spindle abnormalities in vitro. The lowest effective concentration for chromosome congression defects is 40 nM. The dose-response relationship are congruent with the proposed AOP (Shen et al 2005)

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Mixed	Moderate

Studies are available reporting defects of chromosome congression after in vitro exposure of mouse oocytes to spindle poisons [Shen et al., 2005; Eichenlaub-Ritter et al., 2007, Hu et al., 2018]. These studies showed that even exposure to low doses of spindle

poisons, such as nocodazole, induced significant spindle abnormalities that manifested as loss of spindle organization, reduced spindle length at both meiosis I and II and congressional failure among other [Shen et al., 2005; Eichenlaub-Ritter et al., 2007]. Studies on altered chromosome dynamics in human oocytes are scarce. Long-term confocal imaging of chromosome dynamics in 50 human oocytes, collected from women undergoing intracytoplasmic sperm injection showed tri-directional anaphase and other types of chromosomal misalignment in many of them [Haverfield et al., 2017].

Key Event Description

The majority of work for this key event has been conducted in mouse oocytes in vitro. The key event is altered chromosome dynamics at metaphase/anaphase transition. Normal chromosome dynamics refers to the proper alignment and separation of the chromosomes at metaphase and anaphase, respectively. Altered chromosome dynamics refers to the incorrect separation of chromosomes involving an abnormal spindle and a defective cell cycle checkpoint [reviewed in Marchetti et al., 2016].

In oocytes, the meiotic cell division is characterized by unique features with respect to the mitotic process, including: (1) the process by which the meiotic spindle is formed; (2) chromosome organization in bivalents (homologous pairs) with sister kinetochores acting as a functional unit; (3) the role of homologous recombination to ensure proper biorientation and stability of the bivalent structure; (4) the direct entry of oocytes into the second meiotic division, following the first anaphase; and, (5) the lack of chromatin decondensation and formation of the nuclear membrane.

How it is Measured or Detected

Altered chromosome dynamics at metaphase/anaphase is generally assessed by confocal microscopy or enhanced polarizing microscopy on fixed or live cells [Schatten et al., 1985; Shen et al., 2005; Eichenlaub-Ritter et al., 2007; Schuh and Ellenberg, 2007]. Antibodies against centromeric proteins and multicolour fluorescence in situ hybridization (FISH) are useful approaches to follow chromosome congression: for example, distances between kinetochores and spindle midzone are used to evaluate the dynamics of chromosome congression; interkinetochore distances may be measured to verify a correct biorientation [Shen et al. 2005; Eichenlaub-Ritter et al., 2007; Schuh and Ellenberg, 2007; McGuinness et al., 2009; Lane et al., 2012; Mogessie and Schuh, 2017]. A quantitative description of microtubule dynamics and chromosome movement has also been obtained by time-lapse movies of mitotic cells expressing green fluorescence protein (GFP)-conjugate-tubulin [He and Cimini, 2016; Silkworth et al., 2012].

References

Eichenlaub-Ritter U, Winterscheidt U, Vogt E, Shen Y, Tinneberg HR, Sorensen R. 2007. 2-methoxyestradiol induces spindle aberrations, chromosome congression failure, and nondisjunction in mouse oocytes. Biol Reprod 76:784-793.

Haverfield J, Dean NL, Noel D, Remillard-Labrosse G, Paradis V, Kadoch IJ, FitzHarris G. 2017. Tri-directional anaphases as a novel chromosome segregation defect in human oocytes. Hum Reprod 32:1293-1303.

He B, Cimini D. 2016. Using photoactivable GFP to study microtubule dynamics and chromosome segregation. Methods Mol Biol 1413:15-31.

Hu L-L, Zhou X, Zhang H-L, Wu L-L, Tang L-S, Chen L-L, Duan JL. 2018. Exposure to podophyllotoxin inhibits oocyte meiosis by disturbing meiotic spindle formation. Sci Report 8:10145.

Lane SI, Yun Y, Jones KT. 2012. Timing of anaphase-promoting complex activation in mouse oocytes is predicted by microtubule-kinetochore attachment but not by bivalent alignment or tension. Development 139:1947-1955.

Marchetti F, Massarotti A, Yauk CL, Pacchierotti F, Russo A. 2016. The adverse outcome pathway (AOP) for chemical binding to tubulin in oocytes leading to aneuploid offspring. Environ Mol Mutagen 57:87-113.

McGuinness BE, Anger M, Kouznetsova A, Gil-Bernabe AM, Helmhart W, Kudo NR, Wuensche A, Taylor S, Hoog C, Novak B, Nasmyth K. 2009. Regulation of APC/C activity in oocytes by a Bub1-dependent spindle assembly checkpoint. Curr Biol 19:369-380.

Mogessie B, Schuh M. 2017. Actin protects mammalian eggs against chromosome segregation errors. Science 357, eaal1647.

Schuh M, Ellenberg J. 2007. Self-organization of MTOCs replaces centrosome function during acentrosomal spindle assembly in live mouse oocytes. Cell 130:484-498.

Shen Y, Betzendahl I, Sun F, Tinneberg HR, Eichenlaub-Ritter U. 2005. Non-invasive method to assess genotoxicity of nocodazole interfering with spindle formation in mammalian oocytes. Reprod Toxicol 19:459-471.

Silkworth WT, Nardi IK, Paul R, Mogilner A, Cimini D (2012) Timing of centrosome separation is important for accurate chromosome segregation. Mol Cell Biol 23:401-411.

Event: 723: Altered, Chromosome number

Short Name: Altered, Chromosome number

Key Event Component

Process	Object	Action
abnormal chromosome number		increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:106 - Chemical binding to tubulin in oocytes leading to aneuploid offspring	KeyEvent

Stressors

Name
Colchicine
2-Methoxyestradiol
Podophyllotoxin
Nocodazole
Benomyl
Carbendazim
Thiabendazole
Vinblastine sulfate

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
female germ cell

Evidence for Perturbation by Stressor

Colchicine

Ten fold significant increase of hyperhaploid oocytes. 8.6% (30/342) hyperhaploid oocytes vs 0.8% (14/1730) in controls. Oocytes collected aftern natural ovulation, strenghtening the relevance of data for human hazard assessment (Sugawara and Mikamo, 1980)

In Djungarian hamsters, 3 mg/kg Colchicine 5 hours after induction of ovulation induces a significant increase of hyperhaploid oocytes. 11.7% (16/137) hyperhaploid oocytes vs 3.5 in controls (Hummler and Hansmann, 1985).

In mice, 0.25 mg/kg Colchicine significantly increased hyperhaploid oocytes in both young and old female (Tease and Fisher, 1986). In another study, 0.2 mg/kg colchicine at diffrent times from the induction of ovulation (-4 hr to +4 hr) significantly increased hyperhaploid oocyte at all timepoint invegated (Mailhes and Yuan, 1987). This study shows that in preovulatory oocytes the sensitivity window for the induction of aneuploidy is at least 8 hr long. In a subsequent study, a dose-related increase in hyperhaploid oocytes was found (Maihes et al 1988). FInally, another study demonstrated that an aneuploidy induction effectiveness ratio of 10 is observed between administering colchicine orally or by intraperiotoneal injection (Mailhes et al 1990)

2-Methoxyestradiol

Dose related increases in hyperhaploid oocytes after in vitro treatement. The lowest effective tested concentration was 3.75 microM (Eichenlaub-Ritter et al 2007). This study provides evidence that spindle and chromosome congression defects precede the

observation of aneuploid oocytes.

Podophyllotoxin

Administration of 20 mg/kg podophillotoxin at the onset of the first meiotic spindle formation (ie 16 hours before oocyte collection, induced a statistically significant increase in hyperhaploid oocytes from chinese hamsters (Tateno et al 1985)

Nocodazole

In vitro expsoure to nocodazole for one hour during the first meiotic spindel formation induces a statistically significant increase in hyperploid mouse oocytes (Eichenlaub-Ritter and Boll, 1989). Subsequently, a dose-dependent increase in hyperhaploidy oocytes was found (Shen et al 2005); The lowest effective concentration for aneuploidy induction in metaphase II is 40 nM. This paper provides evidence of aneuploid linked to evidence of spindle and chromosome congression defects with a dose response relationship. The study of Sun et al (2005) confirmed the dose-dependent increase in hyperhaploid oocytes and showed that oocytes enclosed in their follicle appear more sensitive than denude oocytes to the aneugenic activity of nocodazole

In vivo, administration of 70 mg/kg nocodazole at the time of the induction of ovulation significantly increased hyperhaploid oocytes while a dose of 35 mg/kg did not (Sun et al 2005)

Benomyl

Administration of benomyl ranging from 500 to 2000 mg/kg per os at the time of the induction of ovulation increased hyperhaploidy mouse oocytes at all doses tested (Mailhes and Aardema, 1992). A saturation of the effect is detected for doses above 1500 mg/kg

Carbendazim

A dose of 1000 mg/kg carbendazin administered per os either 4.5 or 6 hr after induction of ovulation significantly increased hyperhaploidy oocytes in Djungarian hamster (Hummler and Hansmann, 1988). The same dose administered at the time of ovulation induction induced a 4-fold increase in hyperhaploidy oocytes over the control values in Syrian Hamsters (JEffay et al 1996).

Thiabendazole

Thiabendazole was tested in mice at doses ranging 50 to 150 mg/kg. Small but significant increase in hyperhaploid oocytes was found at 100 mg/kg (Mailhes et al 1997)

Vinblastine sulfate

Vinblastine was tested in mice at doses ranging from 0.9 to 9 mg/kg. Significant increses in hyperhaploid oocytes were seen at 0.23 and 0.45 mg/kg (Russo and Pacchierotti, 1988). Higher doses arrested all oocytes at the metaphase I stage, thus, preventing the manifestation of aneuploidy. These results were confirmed in another study (Maihles et al 1993). A study that followed the fate of arrested oocytes, showed that delaying collection of oocytes resulted in a reduction in metaphase I oocytes and a corresponding increase in diploid oocytes (Maihles and Marchetti, 1994).

A study in chinese hamster (Tateno et al 1995) showed that the increase in hyperhaploidy oocytes is similar to what is observed in the mouse with a dose that is 10 times lower.

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
Hamster	Hamster	Moderate	NCBI
Homo sapiens	Homo sapiens	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Mixed	Moderate

Aneuploidy has been measured in many cells types of mammals [Aardema et al., 1998; Mailhes and Marchetti, 1994; 2005; Marchetti et al., 2016], model organisms [Allard et al., 2013; Birchler, 2013] and unicellular organisms [Strome and Plon, 2010]. Therefore, this key event is relevant to all eukaryotic organisms.

Key Event Description

This key event describes the presence of an abnormal number of chromosomes in cells (i.e., aneuploidy) that is different from the haploid number or its multiples.

How it is Measured or Detected

Aneuploidy (i.e., altered chromosome number) is assessed by standard cytogenetic methods that entail the preparation of meiotic or mitotic metaphases to count the number of chromosomes present. Standard methods for assessment in somatic cells have been described and there are OECD test guidelines for cytogenetic analysis of chromosome abnormalities in somatic cells both in vitro [OECD, 2016a] and in vivo [OECD, 2016b]. Although, the detection of aneuploidy for regulatory purposes is not standardized using these approaches, these methods are routinely used in research studies [Aardema et al, 1998]. Aneugens can be detected using the micronucleus assay [OECD, 2016c,d], but these methods are not specific to aneugens. Integration of centromere-specific probes in micronucleus assays enables assessment of aneugenicity using these approaches [Zijno et al., 1996]. Recently, flow cytometry approaches have been developed that are using multiple endpoints to discriminate aneugens from other classes of chemicals [Bryce et al., 2014].

Methods for handling either single oocytes [Tarkowski, 1966] or multiple oocytes [Mailhes and Yuan, 1987] are available. Metaphases are then analyzed under a microscope to count the number of chromosomes. To improve the accuracy of counting, identification of the centromeres can be done using traditional C-banding [Salamanca and Armendares, 1974], fluorescent DNA immunostaining [Leland et al., 2009] or spectral karyotyping [Márquez et al., 1998]. In these studies, the analyzed endpoint is the chromosome number in either second meiotic metaphases or zygotic metaphases. According to a conservative approach, evidence of aneuploidy induction is provided by a statistically significant increase of hyperhaploid metaphases because it cannot be excluded that some hypohaploid metaphases may result from technical artifacts. However, chromosome nondisjunction is expected to produce equal numbers of hyper- or hypohaploid oocytes. Thus, to estimate the total frequency of aneuploid oocytes induced by this mechanism, the frequency of hyperhaploid metaphases is generally doubled. Even this calculation may lead to an underestimate of the absolute aneugenic effect because mechanisms other than nondisjunction, such as chromosome lagging, may produce an excess of hypohaploidies. Indeed, an excess of colchicine-induced hypohaploid oocytes has been reported [Sugawara and Mikamo, 1980].

The cytogenetic analysis of oocytes can also identify the presence of single chromatids originated because of premature sister chromatid separation (PSCS), which is one of the main mechanisms thought to result in aneuploidy in human oocytes [Angell, 1997]. This is has now been demonstrated in mouse oocytes as well [Yun et al., 2014]. The presence of single chromatids in an otherwise normal oocyte can predispose to the induction of aneuploidy during the second meiotic division. In fact, there is one example of a chemical treatment that did not increase aneuploidy in oocytes, but it did so in zygotes because of the presence of PSCS in oocytes [Mailhes et al., 1997].

Oocytes of several rodent species [reviewed in Mailhes and Marchetti, 2005; Pacchierotti et al., 2007] and human oocytes [Pellestor et al., 2005] have been analyzed for assessing aneuploidy. Aneugenicity can also be measured using a C. elegans screening platform for rapid assessment [Allard et al., 2013]. This methodology fluorescently marks aneuploid eggs and embryos.

References

Aardema MJ, Albertini S, Arni P, Henderson LM, Kirsch-Volders M, Mackay JM, Sarrif AM, Stringer DA, Taalman RD. 1998. Aneuploidy: a report of an ECETOC task force. Mutat Res 410:3-79.

Allard P, Kleinstreuer NC, Knudsen TB, Colaiacovo MP. 2013. A C. elegans screening platform for the rapid assessment of chemical disruption of germline function. Environ Health Perspect 121:717-724.

Angell R. 1997. First-meiotic-division non-disjunction in human oocytes. Am J Hum Genet 61:23-32.

Birchler JA. 2013. Aneuploidy in plants and flies: the origin of studies of genomic imbalance. Semin Cell Dev Biol 24:315-319.

Bryce SM, Bemis JC, Mereness JA, Spellman RA, Moss J, Dickinson D, Schuler MJ, Dertinger SD. 2014. Interpreting in vitro micronucleus positive results: simple biomarker matrix discriminates clastogens, aneugens, and misleading positive agents. Environ Mol Mutagen 55:542-555.

Leland S, Nagarajan P, Polyzos A, Thomas S, Samaan G, Donnell R, Marchetti F, Venkatachalam S. 2009. Heterozygosity for a Bub1 mutation causes female-specific germ cell aneuploidy in mice. Proc Natl Acad Sci USA 106:12776-12781.

Mailhes JB, Yuan ZP. 1987. Cytogenetic technique for mouse metaphase II oocytes. Gamete Res 18:77-83.

Mailhes JB, Marchetti F. 1994. Chemically-induced aneuploidy in mammalian oocytes. Mutat Res 320:87-111.

Mailhes JB, Marchetti F. 2005. Mechanisms and chemically-induced aneuploidy in rodent germ cells. Cytogenet Genome Research

111:384-391.

Mailhe JB, Young D, London SN. 1997. 1,2-Propanediol-induced premature centromere separation in mouse oocytes and aneuploidy in one-cell zygotes. Biol Reprod 57:92-98.

Marchetti F, Massarotti A, Yauk CL, Pacchierotti F, Russo A. 2016. The adverse outcome pathway (AOP) for chemical binding to tubulin in oocytes leading to aneuploid offspring. Environ Mol Mutagen 57:87-113.

Márquez C, Cohen J, Munné S. 1998. Chromosome identification in human oocytes and polar bodies by spectral karyotyping. Cytogenet Cell Genet 81:254-258.

Mulla W, Zhu J, Li R. 2014. Yeast: a simple model system to study complex phenomena of aneuploidy. FEMS Microbiol Rev 38:201-212.

OECD. 2016a. *Test No. 473: In Vitro Mammalian Chromosomal Aberration Test*, OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264264649-en.

OECD. 2016b. *Test No. 475: Mammalian Bone Marrow Chromosomal Aberration Test*, OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264264786-en.

OECD. 2016c. *Test No. 474: Mammalian Erythrocyte Micronucleus Test*, OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264264762-en.

OECD. 2016d. *Test No. 487: In Vitro Mammalian Cell Micronucleus Test,* OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264264861-en.

Pacchierotti F, Adler ID, Eichenlaub-Ritter U, Mailhes JB. 2007. Gender effects on the incidence of aneuploidy in mammalian germ cells. Environ Res 104:46-69.

Pellestor F, Anahory T, Hamamah S. 2005. The chromosomal analysis of human oocytes. An overview of established procedures. Human Reprod Update 11:15-32.

Salamanca F, Armendares S. 1974. C bands in human metaphase chromosomes treated by barium hydroxide. Ann Genet 17:135-136.

Strome ED, Plon SE. 2010. Utilizing Saccharomyces cerevisiae to identify aneuploidy and cancer susceptibility genes. Methods Mol Biol 653:73-85.

Sugawara S, Mikamo K. 1980. An experimental approach to the analysis of mechanisms of meiotic nondisjunction and anaphase lagging in primary oocytes. Cytogenet Cell Genet 28:251-264.

Tarkowski AK. 1966. An Air-Drying Method for Chromosome Preparations from Mouse Eggs. Cytogenetic and Genome Research 5:394-400.

Yun Y, Lane SI, Jones KT. 2014. Premature dyad separation in meiosis II is the major segregation error with maternal age in mouse oocytes. Development 141:199-208.

Zijno A, Marcon F, Leopardi P, Crebelli R. 1996. Analysis of chromosome segregation in cytokinesis-blocked human lymphocytes: non-disjunction is the prevalent damage resulting from low dose exposure to spindle poisons. Mutagenesis. 1996 Jul;11(4):335-40

List of Adverse Outcomes in this AOP

Event: 728: Increase, Aneuploid offspring

Short Name: Increase, Aneuploid offspring

Key Event Component

Process	Object	Action
abnormal chromosome number		increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:106 - Chemical binding to tubulin in oocytes leading to aneuploid offspring	AdverseOutcome

Stressors

Name
Colchicine

Biological Context

Level of Biological Organization
Individual

Evidence for Perturbation by Stressor

Colchicine

Dose of 2.0, 3.0 and 4.0 mg/kg colchicine administered at the time of the induction of ovulation significantly increased hyperhaploid zygotes over the control values at all doses tested. Comparison with the data obtained in oocytes under the same experimental conditions suppor the notion that aneuploid oocytes can be fertilized and the chromosome defect transmitted to the embryo

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
Homo sapiens	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Sex Applicability

Sex	Evidence
Female	High

Aneuploid offspring have been measured in mouse and humans, but can occur in any sexually reproducing species.

Key Event Description

An aneuploid offspring is an organism born with an incorrect number of chromosomes (which is present in all of its cells) [reviewed in Marchetti et al., 2016]. In most cases, the aneuploid condition will result in the death of the conceptus at different stages of embryo-fetal development depending on the chromosome involved in the aneuploidy. In humans, most

aneuploid embryos survive until the blastocyst stage and are lost around the time of implantation [Fragouli et al., 2013]; however, a decline in the rate of aneuploidy is already observed between early cleavage stage and the blastocyst stage [Fragouli et al., 2014]. When aneuploid fetuses survive to birth, they will originate offspring affected by aneuploid syndromes, characterized by variable symptoms depending on the specific chromosome involved.

The health consequences of a trisomic condition are well established in both humans and mice. Each of the 19 autosomal trisomies of the mouse has been produced and the survival and phenotype of each trisomy characterized [Epstein, 1988]. Growth retardation is almost invariably present and congenital malformations are frequently detected. Trisomic fetuses generally survive until at least mid-gestation. However, with the exception of trisomy 19 and to a lesser extent trisomy 16 and 18, all die prior to parturition. The precise cause of death of the trisomic embryos is not known. In some instances, it appears to be related to extremely poor embryonic growth and development. Aneuploid mouse zygotes are karyotypically unstable during preimplantation development

leading to a state of chaotic mosaic aneuploidy within the blastocyst [Lightfoot et al., 2006]. In contrast to the survival of trisomic embryos and fetuses until at least mid-gestation, mouse autosomal monosomies are lethal in the pre- or peri-implantation period, with only rare survivors until day 6 of gestation [Magnuson et al., 1985]. Due to dosage compensation mechanisms, aneuploidies of the sex chromosomes in the mouse are viable [Russell, 1976].

Survival data of aneuploidies in humans generally match those in mice: aneuploidies of the sex chromosomes are viable, all autosomal monosomies and most trisomies die before birth, with the exception of trisomy 13, 18 and 21 that, in some cases, survive until shortly after birth or much longer (as in the case of Down syndrome). Even in the case of trisomy 21, the most viable of the human trisomies, an estimated 80% or more fetuses die in utero [Hecht and Hecht, 1987]. Aneuploid conditions compatible with life present a range of adverse health effects from infertility (e.g., Klinefelter syndrome due to XXY karyotype) to severe mental and physical impairment and reduced life span (e.g., Edwards Syndrome due to trisomy 18).

How it is Measured or Detected

Diagnostic laboratories around the world use both phenotypic and molecular approaches to determine whether an individual is aneuploid. Most commonly, tests during pregnancy are used to determine whether a pregnancy is aneuploid [Rink and Norton, 2016]. These include screening tests such as ultrasound examinations [Benacerraf, 2005; Rao and Plat, 2016]; or diagnostic tests during the first or second trimesters, such as chorionic villus sampling [Hogge et al., 1985; Jenkins and Wapner, 1999], amniocentesis [Crandall and Lebher, 1976; Dacu and Wilroy, 1985], and serum markers [Canick et al., 2006]. These are well-established methods that have been used for decades. Recent developments in genomics approaches allow now the diagnosis of an aneuploid pregnancy by detecting fetal cell-free DNA in the blood of the mother [Bianchi et al., 2014; Gil et al., 2017; Sehnert et al., 2011; Valderramos et al., 2016]. When the diagnosis is done after birth, it may be based on the results of a physical exam. For example, children with Down syndrome have distinct facial features that include a flat face, slanting eyes and a small mouth [Fink et al., 1975; Farkas et al., 2002]. A karyotypical analysis of peripheral blood lymphocytes to confirm the presence of the extra chromosome is also conducted.

Regulatory Significance of the AO

Various international regulatory agencies have established policies and practices for the assessment and management of heritable mutagenic hazards. Indeed, heritable effects are an important regulatory endpoint noted by agencies around the world [Yauk et al., 2015a].

The World Health Organization (WHO)/International Programme on Chemical Safety (IPCS) developed a harmonized scheme for mutagenicity testing. In this document the relationship between somatic cell mutagenicity and germ cell risk is summarized as: “For substances that give positive results for mutagenic effects in somatic cells in vivo, their potential to affect germ cells should be considered. If there is toxicokinetic or toxicodynamic evidence that germ cells are actually exposed to the somatic mutagen or its bioactive metabolites, it is reasonable to assume that the substance may also pose a mutagenic hazard to germ cells and thus a risk to future generations.” [Eastmond et al., 2009].

Thus, assessment of heritable mutagenic hazards such as aneuploidy, are an important regulatory endpoint. During drug and chemical development, agents that induce aneuploidy would not be developed further. There is currently not a specific example that can be referenced of a regulatory decision based on this adverse outcome. However, the UK Committee on Mutagenicity of Chemicals in Foods, Consumer Products and the Environment in its 2007 annual report (https://www.gov.uk/government/collections/com-guidance-statements) did recommend that the risk assessment of certain benzimidazoles be conducted solely on the aneugenic properties of these compounds.

The development of AOPs related to mutagenicity in germ cells [Yauk et al., 2015b; 2016] is expected to aid the identification of potential hazards to germ cell genomic integrity and support regulatory efforts to protect population health.

References

Benacerraf BR. 2005. The role of second trimester genetic sonogram in screening for fetal Down Syndrome. Semin Perinatol, 29:386-394.

Bianchi DW, Parker RL, Wentworth J, Madankumar R, Saffer C, Das AF, Craig JA, Chudova DI, Devers PL, Jones KW, Oliver K, Rava RP, Sehnert AJ, CARE Study Group. 2014. DNA sequencing versus standard prenatal diagnosis. N Engl J Med 370:799-808.

Canick JA, Lambert-Messerlian GM, Palomaki GE, Neveus LM, Malone FD, Ball RH, Nyberg DA, Comstock CH, Bukowski R, Saade GR, Berkowits RL, Dar P, Dugoff L, Craigo, SD, Timor-Trisch IE, Carr, SR, Wolfe HM, D’Alton ME. 2006. First and Second Trimester Evaluation of Risk (FASTER) Trial Research Consortium. Comparison of serum markers in first-trimester down syndrome screening. Obstet Gynecol 108:1192-1199.

Crandall BF, Lebherz TB. 1976. Prenatal genetic diagnosis in 350 amniocenteses. Obstet Gynecol, 48:158-162.

Dacus JV, Wilroy RS, Summitt RL, Garbaciak JA, Abdella TN, Spinnato JA, Luthardt FW, Flinn GS, Lewis BA. 1985. Genetic amniocentesis: a twelve years’s experience. Am J Med Genet, 20:443-452.

Eastmond DA, Hartwig A, Anderson D, Anwar WA, Cimino MC, Dobrev I, Douglas GR, Nohmi T, Phillips DH, Vickers. 2009. Mutagenicity testing for chemical risk assessment: update of the WHO/IPCS Harmonized Scheme. Mutagenesis, 24:341-349.

Epstein CJ. 1988. Mouse model systems for the study of aneuploidy. In: Vig BK, Sandberg AA, editors. Aneuploidy, Part B: Induction and Test Systems: Alan R. Liss, Inc. p 9-49.

Farkas LG, Katic MJ, Forrest CR. 2002. Age-related changes in anthropometric measurements in the craniofacial regions and in height in Down's syndrome. J Craniofac Surg 13:614-622.

Fink GB, Madaus WK, Walker GF. 1975. A quantitative study of the face in Down's syndrome. Am J Orthod, 67:540-553.

Fragouli E, Alfarawati S, Spath K, Jaroudi S, Saras J, Enciso M, Wells D. 2013. The origin and impact of embryonic aneuploidy. Hum Genet 132:1001–1013.

Fragouli E, Alfarawati S, Spath K, Wells D. 2014. Morphological and cytogenetic assessment of cleavage and blastocyst stage embryos. Mol Hum Reprod 20:117–126.

Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. 2017. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol. Epub: April 11, 2017. doi: 10.1002/uog.17484.

Hecht F, Hecht BK. 1987. Aneuploidy in humans: dimesions, demography, and dangers of abnormal numbers of chromosomes. In: Vig BK, Sandberg AA, editors. Aneuploidy, Part A: Incidence and Etiology: Alan R. Liss, Inc. p 9-49.

Hogge WA, Schonberg SA, Golbus MS. 1985. Prenatal diagnosis by chorionic villus sampling: lessons of the first 600 cases. Prenat Diagn 5:393-400.

Jenkins TM, Wapner RJ. 1999. First trimester prenatal diagnosis: chorionic villus sampling. Semin Perinatol 23:403-413.

Lightfoot DA, Kouznetsova A, Mahdy E, Wilbertz J, Hoog C. 2006. The fate of mosaic aneuploid embryos during mouse development. Dev Biol 289:384-394.

Magnuson T, Debrot S, Dimpfl J, Zweig A, Zamora T, Epstein CJ. 1985. The early lethality of autosomal monosomy in the mouse. J Exp Zool 236:353-360.

Marchetti F, Massarotti A, Yauk CL, Pacchierotti F, Russo A. 2016. The adverse outcome pathway (AOP) for chemical binding to tubulin in oocytes leading to aneuploid offspring. Environ Mol Mutagen 57:87-113.

Rao R, Platt LD. 2016. Ultrasound screening: status of markers and efficacy of screening for structural abnormalities. Seminal Perinatol 40:67-78.

Rink BD, Norton ME. 2016. Screening for fetal aneuploidy. Semin Perinatol 40:35-43.

Russell LB. 1976. Numerical sex-chromosome anomalies in mammals: Their spontaneous occurrence and use in mutagenesis studies. In: Hollaender A, editor. Chemical Mutagens Principles and Methods for their Detection, vol 4. New York: Plenum Press. p 55-91.

Sehnert AJ, Rhees B, Comstock D, de Feo E, Heilek G, Burke J, Rava RP. 2011. Optimal detection of fetal chromosomal abnormalities by massively parallel DNA sequencing of cell-free DNA from maternal blood. Clin Chem 57:1042-1049.

Valderramos SG, Rao RR, Scibetta EW, Silverman NS, Han CS, Platt LD. 2016. Cell-free DNA screening in clinical practice: abnormal autosomal aneuploidy and microdeletion results. Am J Obstet Gynecol 215:626.e1-626.e10.

Yauk CL, Aardema MJ, Benthem Jv, Bishop JB, Dearfield KL, DeMarini DM, Dubrova YE, Honma M, Lupski JR, Marchetti F, Meistrich ML, Pacchierotti F, Stewart J, Waters MD, Douglas GR. 2015a. Approaches for identifying germ cell mutagens: Report of the 2013 IWGT workshop on germ cell assays. Mutat Res Genet Toxicol Environ Mutagen. 783:36-54.

Yauk CL, Lambert IB, Meek ME, Douglas GR. Marchetti F. 2015b. Development of the adverse outcome pathway "alkylation of DNA in male premeiotic germ cells leading to heritable mutations" using the OECD's users' handbook supplement. Environ Mol Mutagen 56:724-750.

Yauk C, Lamber I, Marchetti F, Douglas G. 2016. Adverse Outcome Pathway on alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations. OECD Series on Adverse Outcome Pathways, No. 3, OECD publishing. http://dx.doi.org/10.1787/5jlsvvxn1zjc-en.

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

[Relationship: 714: Binding, Tubulin leads to Disruption, Microtubule dynamics]

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Chemical binding to tubulin in oocytes leading to aneuploid offspring	adjacent	High	

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Moderate	NCBI
mouse	Mus musculus	High	NCBI
Xenopus laevis	Xenopus laevis	Low	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

This KER has been demonstrated in multiple species including sea urchins, frogs, mice, rats, cows, and human cells in culture.

Key Event Relationship Description

Chemicals that bind to tubulin on colchicine or vinca domain directly interfere with the addition of new tubulin dimers to the microtubules. The result of this process is a net loss of microtubules (i.e., microtubule depolymerization).

Evidence Supporting this KER

Strong based on biological plausibility and available empirical data. There is no uncertainty.

Biological Plausibility

The weight of evidence for this KER is strong. The majority of work for this KER has been derived from research on the prototypical chemical colchicine; however, information is also available for other chemicals such as podophillotoxin, vinblastin, and colcemid. There is high biological plausibility for the binding of colchicine to tubulin leading to microtubule depolymerization, which is one of the most studied chemical interactions with a biological molecule [Margolis and Wilson, 1977; Garland, 1978; Ravelli et al., 2004]. There is extensive understanding of the chemistry of both the binding interactions and the subsequent interference with microtubule dynamics. Depolymerization following colchicine exposure has been measured in frog and mouse eggs, and in human cells, including eggs, in culture [Salmon et al., 1984; Wilson et al., 1984; Ibanez et al., 2003; Liu et al., 2010].

Empirical Evidence

The stoichiometry of colchicine binding to tubulin dimers is well established. Empirical evidence shows that approximately 50% inhibition of microtubule assembly occurs when half of the tubulin dimers are bound by colchicine [Margolis et al., 1980] indicating concordance in the response between the number of bound dimers and degree of depolymerization. Colchicine binding in vitro occurs within minutes of exposure and this timing is concordant with microtubule depolymerization [Margolis and Wilson, 1977]. Colchicine binds to tubulin with an average affinity constant of $10E^{-6}$ to $10E^{-7}$/M at 37 degrees Celsius. The half-life of the binding site is up to 7.5 hours [Hastie 1991; Bhattacharyya et al. 2008] and is concordant with effects on depolymerization and recovery [Margolis and Wilson, 1977].

A concentration of 2.5 µM of colchicine is needed to inhibit microtubule polymerization by 50% [Zavala et al., 1980] and the ability of new chemicals to induce this effect is benchmarked against this value. For example, the IC50 of the tubulin-binding chemical combretastatin A-4 is 1.2 µM [Pettit et al., 1998]. Specificity for tubulin binding is also measured by incubation of tubulin extract with the test chemical in the presence and absence of colchicine. For example, co-incubation of colchicine and the potent microtubule inhibitor podophyllotoxin yields a Ki of 3.3 x 10^{-06} M [Margolis et al., 1980]. Binding constants and resulting effects of depolymerization have been established for at least 20 agents.

Brunner et al. [1991] tested the effects of 10 chemicals on brain tubulin assembly and disassembly in vitro. Tubulin disassembly is monitored for 30-40 minutes following chemical exposure. The 10 chemicals were all compared relative to colchicine as a measure of potency in tubulin binding. Five chemicals (colchicine, vinblastine, thimerosal, thiabendazole, and chloral hydrate) inhibited tubulin polymerization, however, effective concentrations varied by a factor of 30,000 among them. In fact, the concentrations needed to reduce by 30% the steady state of tubulin polymerization were: 0.002 mM for colchicine and vinblastine, 0.03 mM for thimerosal, 0.05 mM for thiabendazole and 60 mM for chloral hydrate. Furthermore, except for chloral hydrate, these compounds also induced a dose-dependent decline in polymerization velocity. Wallin and Hartley-Asp [1993] tested the same 10 chemicals for effects on the assembly of bovine microtubules and reported similar results to those of Brunner et al [1991] although some differences in potencies were also noted.

Salmon et al. [1984] showed that in sea urchins, injection of colchicine or colcemid at final intracellular concentrations of 0.1-3.0 mM leads to a rapid microtubule depolymerization throughout the central spindle and aster. Microtubule concentration in the central half-spindle decreased exponentially to 10% of its initial value within ~20 s. For both colchicine and colcemid, the rate of microtubule depolymerization below 0.1 mM was concentration dependent. In addition, they show that increasing doses of colchicine lead to reductions in the amount of time necessary to detect microtubule depolymerization. As a control, lumicolchicine (which does not bind to tubulin with high affinity) had no effect on microtubule polymerization at intracellular concentrations of 0.5 mM.

Uncertainties and Inconsistencies

No apparent uncertainties or inconsistencies. This KER is biologically plausible and broadly accepted. Indeed, in vitro assays to measure tubulin depolymerization are well standardized and represent the gold standard to determine whether a chemical is binding to tubulin.

Quantitative Understanding of the Linkage

As described above, the quantitative relationship is well established for colchicine, and other chemicals are benchmarked against this chemical [Brunner et al., 1991]. Microtubule assembly is inhibited by approximately 50% when half of the tubulin dimers are bound by colchicine [Margolis et al., 1980], and a concentration of 2.5 µM of colchicine is needed to inhibit microtubule polymerization by 50% [Zavala et al., 1980].

Response-response relationship

Microtubule assembly is inhibited by approximately 50% when half of the tubulin dimers are bound by colchicine [Margolis et al., 1980], and a concentration of 2.5 µM of colchicine is needed to inhibit microtubule polymerization by 50% [Zavala et al., 1980].

Time-scale

Colchicine binds slowly to tubulin, in contrast to Combretastatin A4, which binds in a relatively fast, temperature-dependent manner. The rate of Colchicine binding has a rate constant of ~10^2 M^{-1} s^{-1} as determined by an isotopic labeling technique [Gaarland D.L. 1978]. Hovever, colchicine dissociates from tubulin over 100 times slower than combretastatin A-4, with a half.life of 405 min at 37 °C, compared to 3.6 min of CA4 [Lin et al. 1989].

Known modulating factors

Microtubules assembled *in vitro* contain several minor protein components that have been referred to as microtubule-associated proteins (MAPs). Several of these proteins are believed to play a role in the microtubule assembly process [Kakiu & Sato, 2016]. MAPs have been shown to inhibit colchicine binding to tubulin in a competitive manner. In contrast, Mg^{2+}, which also induces microtubule assembly *in vitro*, had no effect on colchicine binding to tubulin [Nunez J et al. 1978].

Known Feedforward/Feedback loops influencing this KER

To our knowledge, there are no feedback loops influencing this KER.

References

Bhattacharyya B, Panda D, Gupta S, Banerjee M. 2008. Anti-mitotic activity of colchicine and the structural basis for its interaction with tubulin. Med Res Rev 28:155-183.

Brunner M, Albertini S, Würgler FE. 1991. Effects of 10 known or suspected spindle poisons in the in vitro porcine brain tubulin assembly assay. Mutagen 6:65-70.

Garland DL. 1978. Kinetics and mechanism of colchicine binding to tubulin: Evidence for ligand-induced conformational change. Biochemistry 17:4266–4272.

Hastie SB. 1991. Interaction of colchicine with tubulin. Pharmacol Ther 51:377-401.

Kakui Y, Sato M. 2016. Differentiating the roles of microtubule-associated proteins at meiotic kinetochores during chromosome segregation. Chromosoma 125:309-320.

Ibanez E, Albertini DF, Overstrom EW. 2003. Demecolcine-induced oocyte enucleation for somatic cell cloning: Coordination

between cell-cycle egress, kinetics of cortical cytoskeletal interactions, and second polar body extrusion. Biol Reprod 68:1249–1258.

Linn CM, Ho HH, Pettit GR, Hamel E. 1989. Antimitotic natural products combretastatin A-4 ad combretastatin A: studies on the mechanism of their inhibition of the binding of colchicine to tubulin. Biochemistry 28:6984-6991.

Liu S, Li Y, Feng HL, Yan JH, Li M, Ma SY, Chen ZJ. 2010. Dynamic modulation of cytoskeleton during in vitro maturation in human oocytes. Am J Obstet Gynecol 203:151.e151–157.

Nunez J, Fellous A, Francon J, Lennon AN. 1978. Competitive inhibition of colchicine binding to tubulin by microtubule-associated proteins. Proc Natl Acad Sci USA 76:86-90.

Margolis RL, Wilson L. 1977. Addition of colchicine-tubulin complex to microtubule ends: the mechanism of substoichiometric colchicine poisoning. Proc Natl Acad Sci U S A 74:3466-3470.

Margolis RL, Rauch CT, Wilson L. 1980. Mechanism of colchicine-dimer addition to microtubule polymerization mechanism. Biochemistry 19:5550-5557.

Pettit GR, Toki B, Herald DL, Verdier-Pinard P, Boyd MR, Hamel E, Pettit RK. 1998. Antineoplastic agents. 379. Synthesis of phenstatin phosphate. J Med Chem 41:1688-1695.

Ravelli RB, Gigant B, Curmi PA, Jourdain I, Lachkar S, Sobel A, Knossow M. 2004. Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. Nature 428:198–202.

Salmon ED, McKeel M, Hays T. 1984. Rapid rate of tubulin dissociation from microtubules in the mitotic spindle in vivo measured by blocking polymerization with colchicine. J Cell Biol 99:1066–1075.

Wallin M, Hartley-Asp B. 1993. Effects of potential aneuploidy inducing agents on microtubule assembly in vitro. Mutat Res 287:17-22.

Wilson L, Miller HP, Pfeffer TA, Sullivan KF, Detrich HW,3. 1984. Colchicine-binding activity distinguishes sea urchin egg and outer doublet tubulins. J Cell Biol 99:37–41

Relationship: 715: Disruption, Microtubule dynamics leads to Disorganization, Meiotic Spindle

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Chemical binding to tubulin in oocytes leading to aneuploid offspring	adjacent	Moderate	

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Moderate	NCBI
mouse	Mus musculus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult	High

Sex Applicability

Sex	Evidence
Mixed	Moderate

Data were produced in sea urchins, mice and human eggs and embryos. This KER should be applicable to any eukaryotic organism.

Key Event Relationship Description

Spindle organization and function requires normal microtubule dynamics. When microtubule polymerization is affected (i.e., depolymerization), spindle organization and function is impaired.

Evidence Supporting this KER

Moderate.

Biological Plausibility

The weight of evidence for this KER is moderate. Microtubule polymerization is critical for the appropriate functioning of the spindle. Mitotic and meiotic spindles differ in how they are assembled. In mitotic cells, spindle organization is controlled by centrioles [Walczak and Heald, 2008; Wadsworth et al., 2011; Wittman et al., 2011]. However, centrioles are absent in mammalian oocytes [Manandhar et al., 2005] and meiotic spindle is organized by multiple microtubule organizing centers (MTOCs). Gradually, MTOCs coalesce and surround the chromosomes and subsequently elongate in a typical barrel-shape bipolar spindle [Schuh and Ellenberg, 2007; Clift and Schuh, 2015], similar to the mitotic spindle. Assembly, elongation and function of the spindle requires proper microtubule dynamics. If microtubules become depolymerized, it affects the structural integrity of the spindle resulting in abnormal spindles that are characterized by reduction in microtubule density, loss of barrel shape, mono- or multi-polar spindle, and reduced distance between the poles [Ibanez et al., 2003; Shen et al., 2005; Eichenlaub-Ritter et al., 2007; Xu et al., 2012]. The normal biology underlying the critical role of proper microtubule polymerization for the appropriate structure and function of spindle is well established, and it is widely understood that chemicals that alter microtubule dynamics cause spindle disorganization [Manandhar et al., 2005; Schuh and Ellenberg, 2007].

Empirical Evidence

Several papers have explored the temporal and incidence relationship between microtubule depolymerization and appearance of spindle abnormalities, providing empirical evidence to support this KER in a variety of species. For example, Salmon et al. [1984] showed that within 20 seconds of colchicine or colcemid administration in sea urchin embryos, microtubule depolymerization occurs. This leads to spindle abnormalities at the same concentration that occurs a few minutes later. In Liu et al. [2010], in vitro culturing of human oocytes in the presence of 10 µM colchicine prevented microtubule polymerization and because of this, meiotic spindle did not form (i.e., exposure led to failure to progress to meiosis II supporting temporal concordance). Ibanez et al. [2003] showed that 1 µM colcemid caused microtubule depolymerization that led to smaller spindles and lower microtubule density in mouse oocytes within 15 minutes of exposure, demonstrating temporal and incidence concordance.

Uncertainties and Inconsistencies

There are not a lot of studies that have explored these two events within the same experiment. Thus, the empirical evidence is not based on a large number of papers. However, the papers that are available are of sound experimental design that address both time and incidence relationships, and span three species.

Quantitative Understanding of the Linkage

Limited quantitative understanding. Data from Salmon et al. [1984] established a dose-response relationship for microtubule depolymerization in mitotic sea urchin embryo cells, but similar quantitative data are not available for spindle disorganization.

Response-response relationship

There are detailed dose-response relationships for microtubule depolymerisation by tubulin binders obtained using acellular tubulin polymerization assays [Zavala et al., 1980; Hamel and Lin, 1981; Verdier-Pinard et al., 1998; Miller and Wilson, 2010]. The rate of depolymerisation has been also measured in whole mitotic cells of sea urchin embryos after microinjection of different doses of colchicine or colcemid, in the range 0.01-5 mM [Salmon et al., 1984]. Comparable data are not available for mammalian oocytes. In addition, no quantitative dose-response relationship has been obtained for spindle disorganization in oocytes treated with tubulin binding chemicals. This lack of data does not allow modelling a response-response relationship between disruption of microtubule dynamics (KEupstream) and spindle disorganization (KEdownstream).

Time-scale

In sea urchin embryo cells microinjected with colchicine concentrations equal to or higher than 0.1 mM, complete depolymerization of non-kinetochore spindle microtubules (KEupstream) is reached in about 20 seconds, corresponding to a depolymerization rate of about 180-992 dimers per second [Salmon et al., 1984]. The order of magnitude of these values corresponds to the fastest rates of tubulin dissociation reported in various acellular systems [Fan'ell et al., 1983]. However, possible modifying factors of the above rates are suggested in the cells (e.g., calcium concentration), conditions that are not reproducible in acellular systems.

In vitro exposure of mouse oocytes to 67 µM nocodazole causes a gradual disorganization of the spindle (KEdownstream), which is completed within 15 min [Xu et al., 2012]. In spite of the limited amount of data on the kinetics of spindle disorganization (KEdownstream) and the further limitation that dysruption of microtubule dynamics (KEupstream) and spindle disorganization (KEdownstream) were not analyzed in the same biological systems, it can be noted that the time-scale in the KEdownstream is coherent with the time-scale of the KEupstream.

Known modulating factors

Due to the heterogeneity of the experimental approaches used to measure dysruption of microtubule dynamics (KEupstream) and spindle disorganization (KEdownstream) it is not feasible to identify modulating factors acting in this KER.

Known Feedforward/Feedback loops influencing this KER

To our knowledge, there are no feedback loops influencing this KER.

References

Clift D, Schuh M. 2015. A three-step MTOC fragmentation mechanism facilitate bipolar spindle assembly in mouse oocytes. Nat Commun 6:7217.

Eichenlaub-Ritter U, Winterscheidt U, Vogt E, Shen Y, Tinneberg HR, Sorensen R. 2007. 2-methoxyestradiol induces spindle aberrations, chromosome congression failure, and nondisjunction in mouse oocytes. Biol Reprod 76:784–793.

Fan'ell KW, Himes RH, Jordon MA,Wilson L. 1983. On the nonlinear relationship between the initial rates of dilution induced microtubule disassembly and the initial free subunit concentration. J Biol Chem 258:14148-14156.

Hamel E, Lin CM. 1981. Stabilization of the colchicine-binding activity of tubulin by organic acids. Biochim Biophys Acta 675:226-231.

Ibanez E, Albertini DF, Overstrom EW. 2003. Demecolcine-induced oocyte enucleation for somatic cell cloning: Coordination between cell-cycle egress, kinetics of cortical cytoskeletal interactions, and second polar body extrusion. Biol Reprod 68:1249–1258.

Liu S, Li Y, Feng HL, Yan JH, Li M, Ma SY, Chen ZJ. 2010. Dynamic modulation of cytoskeleton during in vitro maturation in human oocytes. Am J Obstet Gynecol 151:e1–7.

Manandhar G, Schatten H, Sutovsky P. 2005. Centrosome reduction during gametogenesis and its significance. Biol Reprod 72:2-13.

Miller HP, Wilson L. 2010. Chapter 1 - Preparation of Microtubule Protein and Purified Tubulin from Bovine Brain by Cycles of Assembly and Disassembly and Phosphocellulose Chromatography. In: Leslie W, John JC, editors. Methods Cell Biol: Academic Press. p 2-15.

Salmon ED, McKeel M, Hays T. 1984. Rapid rate of tubulin dissociation from microtubules in the mitotic spindle in vivo measured by blocking polymerization with colchicine. J Cell Biol 99:1066-1075.

Schuh M, Ellenberg J. 2007. Self-organization of MTOCs replaces centrosome function during acentrosomal spindle assembly in live mouse oocytes. Cell 130:484-498.

Shen Y, Betzendahl I, Sun F, Tinneberg HR, Eichenlaub-Ritter U. 2005. Non-invasive method to assess genotoxicity of nocodazole interfering with spindle formation in mammalian oocytes. Reprod Toxicol 19:459-471.

Verdier-Pinard P, Lai JY, Yoo HD, Yu J, Marquez B, Nagle DG, Nambu M, White JD, Falck JR, Gerwick WH, Day PW, Hamel E. 1998. Structure-activity analysis of the interaction of curacin A, the potent colchicine site antimitotic agent, with tubulin and effects of analogs on the growth of MCF-7 breast cancer cells. Mol Pharmacol 53:62-76.

Wadsworth P, Lee WL, Murata T, Baskin TI. 2011. Variations on a theme: spindle assembly in diverse cells. Protoplasma 248:439-446.

Walczak CE and R Heald. 2008. Mechanisms of mitotic spindle assembly and function. Int. Rev Cytol 265:111-158.

Wittman T, Hyman A, Desai A. 2011. The spindle: a dynamic assembly of microtubules and motors. Nat Cell Biol 3:e28-234.

Xu XL, Ma W, Zhu YB, Wang C, Wang BY, An N, An L, Liu Y, Wu ZH, Tian JH. 2012. The microtubule-associated protein ASPM regulates spindle assembly and meiotic progression in mouse oocytes. PLoS One 7:e49303.

Zavala F, Guenard D, Robin JP, Brown E. 1980. Structure--antitubulin activity relationship in steganacin congeners and analogues. Inhibition of tubulin polymerization in vitro by (+/-)-isodeoxypodophyllotoxin. J Med Chem 23:546-549.

Relationship: 737: Disorganization, Meiotic Spindle leads to Altered, Meiotic chromosome dynamics

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Chemical binding to tubulin in oocytes leading to aneuploid offspring	adjacent	Moderate	

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Female	Moderate

Although this KER has only been measured in mouse oocytes, the process of meiosis, spindle formation and chromosome congression in eggs is thought to be similar across mammalian species.

Key Event Relationship Description

Incorrect spindle organization refers to lack of the bipolar organization of the spindle within the cell. This bipolar organization is required to assure that chromosomes will align correctly to the metaphase plate prior to equal division between the daughter cells. Alternatively, incorrect spindle formation can lead to shorter spindle fibers and/or incorrect length of these fibers, which leads to chromosome misalignment.

In this KER, chemicals that cause spindle disorganization lead to altered meiotic chromosome dynamics. The relationship between spindle disorganization and altered chromosome dynamics can occur in both somatic and in germ cells; however, this relationship focuses on female meiotic chromosomes because of the differences in how the meiotic spindle is assembled in oocytes with respect to other cell types (i.e., lack of centrioles and dependency on microtubule organizing centers). Interestingly, some studies investigating the effects of protein deficiencies in mouse oocytes provide direct evidence of the events involved in the KER [McGuinnes et al., 2009; Ou et al., 2010; Baumann et al., 2017]. For example, targeting deletion of Bub1 in mouse oocytes caused dysregulation of spindle assembly and leads to defective chromosome congression [McGuinness et al., 2009]. After depletion of p38a in mouse oocytes, a MTCO component, aberrant spindle organization, including defective or multipolar spindles are more than 3 times more frequent than in control mice, while there is an 8-fold increase in chromosome congression defects [Ou et al., 2010]. Finally, in a study carried out using an oocyte conditional pericentrin knockout mouse model and live cell imaging, alterations in spindle size have been observed, together with delay in meiotic spindle formation following in vitro culture of cumulus-enclosed oocytes. These abnormalities were associated with a significant increase in the number of unattached kinetochores and merotelic attachments, as well as, an increase in misaligned and uncongressed chromosomes [Baumann et al., 2017].

Evidence Supporting this KER

Moderate, based on strong biological plausibility and weak emprical evidence.

Biological Plausibility

The weight of evidence for this KER is moderate. It is well understood that the proper organization of the meiotic spindle is necessary in order for chromosomes to correctly align. This process has been extensively described in the literature. For a recent comprehensive review on this topic, please see Bennabi et al. [2016].

Empirical Evidence

Although it is very common to measure spindle abnormalities (i.e., spindle disorganization), few studies have examined meiotic chromosome dynamics. Thus, there are insufficient empirical data examining the concordance between spindle abnormalities and chromosome dynamics.

However, two in vitro studies on mouse eggs have investigated spindle abnormalities and chromosome congression defects within individual studies (i.e., both endpoints measured). These studies used nocodazole and 2-methoxyestradiol to demonstrate that there is a temporal and dose-response related consistency among the events; i.e., downstream KEs are occurring at higher doses and later time points than upstream KEs [Shen et al., 2005; Eichenlaub-Ritter et al., 2007]. Further evidence supporting this KER has been collected in somatic cells, especially in vitro (reviewed in Silkworth and Cimini, 2012).

Uncertainties and Inconsistencies

There is not extensive empirical data this KER, however, the available data does not show inconsistencies.

Quantitative Understanding of the Linkage

Although there are no inconsistent results reported, it is important to note that very few studies have measured chromosome dynamics in oocytes in general. Thus, there is a large amount of uncertainty surrounding the qualitative and quantitative association

between these two endpoints.

Response-response relationship

As noted above, very few studies have examined both spindle abnormalities (KEupstream) and altered chromosome dynamids (KEdownstream) under the same experimental conditions, especially in oocytes. In addition, spindle abnormalities (KEupstream) and altered chromosome dynamics (KEdownstream) in oocytes treated with tubulin binding chemicals have not been quantitated by detailed dose-response relationships. Thus, the dataset is too limited to allow defining a response-response relationship between spindle abnormalities (KEupstream) and altered chromosome dynamics (KEdownstream). A study on mouse oocytes treated in vitro with nocodazole [Shen et al., 2005] showed that KEdownstream occurred at a dose higher than doses inducing KEustream, suggesting that a certain level of spindle abnormalities (KEupstream) is to be reached before altered chromosome dynamics (KEdownstream) occur, but data are too limited to draw a firm conclusion on the shape of the response-response relationship.

Time-scale

Spindle formation (KEupstream) and chromosome congression on the metaphase plate (KEdownstream) are highly dynamic processes. In mouse oocytes, the first meiotic spindle is assembled in 3–4 h, and 3 more hours are needed for it to migrate to the cortex [Wei et al., 2018]. During the following 2 hours, chromosome congress at the spindle equator by a trial and error process connecting kinetochores with kinetochore fibres. The establishment of complete and correct connections is monitored by checkpoint mechanisms that control anaphase triggering. Live imaging studies of oocytes treated with tubulin binding chemicals are not available that could allow the timing of changes in KEdownstream in relation to the start of changes in KEupstream. However, live imaging studies under impaired spindle assembly conditions [Yi et al., 2019] suggest that spindle disorganization (KEupstream) induces altered chromosome dynamics (KEdownstream) in a matter of minutes. Alterations may last for hours, if spindle damage is sustained by continuous chemical exposure. In fact, anaphase onset may be delayed by hours when oocytes are exposed to spindle disrupting chemicals [Mailhes et al., 1993; Mailhes and Marchetti, 1994].

Known modulating factors

Due to the lack of solid evidence about the response-response relationship modulating factors cannot be identified in this KER.

Known Feedforward/Feedback loops influencing this KER

To our knowledge, there are no feedback loops influencing this KER.

References

Baumann C, Wang X, Yang L, Viveiros MM. 2017. Error-prone meiotic division and subfertility in mice with oocyte-conditional knockdown of pericentrin. J Cell Sci 130:1251-1262.

Eichenlaub-Ritter U, Winterscheidt U, Vogt E, Shen Y, Tinneberg HR, Sorensen R. 2007. 2-methoxyestradiol induces spindle aberrations, chromosome congression failure, and nondisjunction in mouse oocytes. Biol Reprod 76:784–793.

Mailhes JB, Marchetti F. 1994. Chemically-induced aneuploidy in mammalian oocytes. Mutat Res 320:87-111.

Mailhes JB, Aardema MJ, Marchetti F. 1993. Investigation of aneuploidy induction in mouse oocytes following exposure to vinblastine-sulfate, pyrimethamine, diethylstilbestrol diphosphate, or chloral hydrate. Environ Mol Mutagen 22:107–114.

McGuinness BE, Anger M, Kouznetsova A, Gil-Bernabe AM, Helmhart W, Kudo NR, Wuensche A, Taylor S, Hoog C, Novak B, Nasmyth K. 2009. Regulation of APC/C activity in oocytes by a Bub1-dependent spindle assembly checkpoint. Curr Biol 19:369-380.

Ou XH, Li S, Xu BZ, Wang ZB, Quan S, Li M, Zhang QH, Ouyang YC, Schatten H, Xing FQ, Sun QY. 2010. p38alpha MAPK is a MTOC-associated protein regulating spindle assembly, spindle length and accurate chromosome segregation during mouse oocyte meiotic maturation. Cell Cycle 9:4130-4143

Shen Y, Betzendahl I, Sun F, Tinneberg HR, Eichenlaub-Ritter U. 2005. Non-invasive method to assess genotoxicity of nocodazole interfering with spindle formation in mammalian oocytes. Reprod Toxicol 19:459–471.

Silkworth WT, Cimini D. 2012. Transient defects of mitotic spindle geometry and chromosome segregation errors. Cell Div 7:19.

Yi Z-Y, Liang Q-X, Meng T-G, Li J, Dong M-Z, Hou Y, Ouyang Y-C, Zhang C-H, Schatten H, Sun Q-Y, Qiao J, Qian WP. 2019. PKCβ1 regulates meiotic cell cycle in mouse oocyte. Cell Cycle, DOI: 10.1080/15384101.2018.1564492

Wei Z, Greaney J, Zhou C, Homer H. 2018. Cdk1 inactivation induces post-anaphase-onset spindle migration and membrane protrusion required for extreme asymmetry in mouse oocytes. Nature Comm 9:4029. DOI: 10.1038/s41467-018-06510-9.

Relationship: 738: Altered, Meiotic chromosome dynamics leads to Altered, Chromosome number

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Chemical binding to tubulin in oocytes leading to aneuploid offspring	adjacent	Low	

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	Moderate	NCBI

Sex Applicability

Sex	Evidence
Female	Moderate

Although this KER has only been measured in mouse oocytes, the process of meiosis, spindle formation and chromosome congression in eggs is thought to be similar across mammalian species.

Key Event Relationship Description

Chromosome dynamics refers to the ability of chromosomes to congress at the metaphase plate before segregation and attach in an amphitelic orientation [Mailhes and Marchetti, 2010]. Amphitelic refers to the proper attachment of homologous chromosomes to a bipolar spindle and their orientation to opposite poles. Each daughter cell is then expected to receive one chromosome (composed of two chromatids), resulting in a haploid state. Cells have the SAC that monitors chromosome dynamics and should prevent anaphase from occurring in the presence of misaligned chromosomes, however, especially in oocytes, the SAC is not always able to arrest meiotic progression in the presence of misaligned chromosomes.

In this KER, alterations in chromosome dynamics lead to incorrect congression and alignment. In addition, the SAC fails to prevent chromosome segregation, resulting in an aneuploid cell.

Evidence Supporting this KER

Weak.

Biological Plausibility

The weight of evidence for this KER is weak. The mechanistic aspects of chromosome dynamics are well understood [Bennabi et al., 2016; Touati and Wassmann, 2016]. It is broadly understood that correct chromosome alignment is required for to produce an egg with the correct number of chromosomes and that the probability of an aneuploid egg is increased when chromosomes fail to align correctly. However, chromosome misalignment does not always lead to subsequent errors in chromosome segregation. This may be due in part to the important role of the SAC in blocking chromosome segregation when chromosomes are not correctly aligned [Amon, 1999; Musacchio and Salmon, 2007; Polanski, 2013; Musacchio, 2015]. At this time, there is not complete mechanistic understanding of every step in this process.

Empirical Evidence

There are insufficient empirical data examining the concordance between chromosome dynamics and generation of aneuploidy oocytes because very few studies have examined chromosome dynamics in these cells.

Two in vitro studies have investigated chromosome congression defects and aneuploidy in mouse oocytes. Using nocodazole and 2-methoxyestradiol these studies demonstrated that there is a temporal and dose-response related consistency among the events; i.e., downstream KEs are occurring at higher doses and later time points than upstream KEs [Shen et al., 2005; Eichenlaub-Ritter et al., 2007]. Specifically, exposure of mouse oocytes to increasing concentrations of 2-methoxyestradiol demonstrates: 1) abnormal spindle formation beginning at 3.75 uM (53% of cells), and increasing to 75% at 5 uM, and 100% by 7.5 uM; 2) hyperploidy occurring at 6%, 23% and 100% at 3.75, 5 and 7.5 uM, respectively; and 3) abnormal spindle forming as early as 9 hr, and aneuploidy arising by 16 hrs. Similarly, in Shen et al. (2005), mouse oocytes exposed to nocodazole showed abnormal chromosome alignment in 9%, 22% and 23% of oocytes, which is concordant with a 0%, 3% and 10% increase in hyperploid oocytes at 20nM, 30nM and 40nM, respectively. Moreover, alignment errors were measured at 13 hr, whereas aneuploidy was found at 16 hr. Both of these studies demonstrate that errors in chromosome alignment occur earlier and at higher rates than aneuploidy in eggs. A causal correlation between chromosome misalignment and generation of aneuploid oocytes has been reported after exposure to bisphenol A [Hunt et al., 2003].

Additional evidence is coming from some studies investigating the effects of protein deficiencies in mouse oocytes, and reporting a relationship between altered chromosome dynamics and aneuploidy [Mc Guinness et al., 2009; Ou et al., 2010; Baumann et al., 2017]. Targeting deletion of Bub1 in mouse oocytes leads to defective chromosome segregation and aneuploidy is monitored for

the whole chromosome set by the multicoloured SKY FISH approach [Mc Guinness et al. 2009]. After depletion in mouse oocytes of the MTOC component p38a chromosome congression defects are 8 times more frequent than in controls, and under these conditions the incidence of aneuploid oocytes is about 8-fold higher [Ou et al., 2010]. In a study carried out using a oocyte conditional pericentrin knockout mouse model and live cell imaging, it has been demonstrated that unattached kinetochores, merotelic attachments, misaligned and uncongressed chromosomes are significantly increased and this is causing an increase of ploidy defects [Baumann et al., 2017]. Although based on genetic models, these data provide a direct evidence of the mechanisms involved in this KER.

Uncertainties and Inconsistencies

Although there are no inconsistent results reported, it is important to note that very few studies have measured chromosome dynamics and induction of aneuploidy in oocytes.

Quantitative Understanding of the Linkage

There is a large amount of uncertainty surrounding the qualitative and quantitative association between these two endpoints.

Response-response relationship

Data are available on the dose-response relationship for aneuploidy induction in oocytes (KEdownstream) treated with colchicine [Mailhes et al., 1988; Mailhes et al., 1990], vinblastine [Russo and Pacchierotti, 1988; Mailhes et al., 1993] or 2-methoxyestradiol [Eichenlaub-Ritter et al., 2007], which are consistent with the threshold relationship established in mitotic cells [Elhajouji et al., 2011]. Unfortunately, dose-effect relationships have not been established for chromosome dynamics alterations (KEupstream) at the first meiotic division. Thus, it is not possible to establish the shape of the response-response relationship between chromosome dynamics alteratiions (KEupstream) and altered chromosome nubmer in oocytes (KEdownstream).

Time-scale

As noted before, chromosome dynamics on the metaphase plate of oocytes may last a few hours before anaphase onset. The first meiotic anaphase lasts about 25 min equally distributed between anaphase-1, characterized by increased spindle length and movement of chromosomes towards the poles, and anaphase-2 at the end of which chromosomes reach the poles and aggregate into condensed clusters [Wei et al., 2018]. Thus, it is expected that alterations of chromosome number in the oocyte (KEdownstream) would lag alterations of meiotic chromosome dynamics (KEupstream) by hours, although no studies have been carried out until now to specifically address the time-scale of events linking chromosome dynamics alteratiions (KEupstream) and altered chromosome nubmer in oocytes (KEdownstream).

Known modulating factors

Due to the lack of information about the shape of the response-response relationship, modulating factors cannot be identified in this KER.

Known Feedforward/Feedback loops influencing this KER

In mitotic and meiotic cells, anaphase onset and ensuing chromosome distribution is under checkpoint control that may delay anaphase onset until chromosomes are correctly aligned on the spindle equator, as signaled by specific molecular events [Nagaoka et al., 2012; Musacchio et al., 2015; Webster and Schuh, 2017]. Although the SAC in mammalian oocytes is deemed to be more tolerant to the presence of unaligned chromosomes, its role in preventing aneuploidy is proven in genetically modified or silenced systems [Mailhes and Marchetti 2010]. These checkpoint and signaling mechanisms therefore are expected to act as feedback loops, which may influence the time-scale of the KER between KEupstream (altered chromosome dynamics) and altered chromosome number in the oocyte (KEdownstream).

References

Amon A. 1999. The spindle checkpoint. Curr Opin Genet Dev 9:69-75.

Baumann C, Wang X, Yang L, Viveiros MM. 2017. Error-prone meiotic division and subfertility in mice with oocyte-conditional knockdown of pericentrin. J Cell Sci 130:1251-1262.

Bennabi I, Terret ME, Verlhac MH. 2016. Meiotic spindle assembly and chromosome segregation in oocytes. J Cell Biol 215:611-619.

Eichenlaub-Ritter U, Winterscheidt U, Vogt E, Shen Y, Tinneberg HR, Sorensen R. 2007. 2-methoxyestradiol induces spindle aberrations, chromosome congression failure, and nondisjunction in mouse oocytes. Biol Reprod 76:784–793.

Elhajouji A, Lukamowicz M, Cammerer Z, Kirsch-Volders M. 2011. Potential thresholds for genotoxic effects by micronucleus scoring. Mutagenesis 26:199-204.

Hunt PA, Koehler KE, Susiarjo M, Hodges CA, Ilagan A, Voigt RC, Thomas S, Thomas BF, Hassold TJ. 2003. Bisphenol a exposure causes meiotic aneuploidy in the female mouse. Curr Biol 13:546-553.

Mailhes JB, Preston RJ, Yuan ZP, Payne HS. 1988. Analysis of mouse metaphase II oocytes as an assay for chemically induced aneuploidy. Mutat Res 198:145–152.

Mailhes JB, Yuan ZP, Aardema MJ. 1990. Cytogenetic analysis of mouse oocytes and one-cell zygotes as a potential assay for heritable germ cell aneuploidy. Mutat Res 242:89–100.

Mailhes JB, Aardema MJ, Marchetti F. 1993. Investigation of aneuploidy induction in mouse oocytes following exposure to vinblastine-sulfate, pyrimethamine, diethylstilbestrol diphosphate, or chloral hydrate. Environ Mol Mutagen 22:107–114.

Mailhes JB, Marchetti F. 2010. Advances in understanding the genetic causes and mechanisms of female germ cell aneuploidy. Exp Rev Obst Gyn 5:687–706.

McGuinness BE, Anger M, Kouznetsova A, Gil-Bernabe AM, Helmhart W, Kudo NR, Wuensche A, Taylor S, Hoog C, Novak B, Nasmyth K. 2009. Regulation of APC/C activity in oocytes by a Bub1-dependent spindle assembly checkpoint. Curr Biol 19:369-380.

Musacchio A. 2015. The molecular biology of spindle assembly checkpoint signaling dynamics. Curr Biol 25:R1002-R1018.

Musacchio A, Salmon ED. 2007. The spindle-assembly checkpoint in space and time. Nat Rev Mol Cell Biol 2007; 8:379-93.

Nagaoka SI, Hassold TJ, Hunt PA. 2012. Human aneuploidy: Mechanisms and new insights into an age-old problem. Nat Rev Genet 13:493–504.

Ou XH, Li S, Xu BZ, Wang ZB, Quan S, Li M, Zhang QH, Ouyang YC, Schatten H, Xing FQ, Sun QY. 2010. p38alpha MAPK is a MTOC-associated protein regulating spindle assembly, spindle length and accurate chromosome segregation during mouse oocyte meiotic maturation. Cell Cycle 9:4130-4143.

Polanski Z. 2013. Spindle assembly checkpoint regulation of chromosome segregation in mammalian oocytes. Reprod Fertil Dev 25:472-483.

Russo A, Pacchierotti F. 1988. Meiotic arrest and aneuploidy induced by vinblastine in mouse oocytes. Mutat Res 202:215–221.

Shen Y, Betzendahl I, Sun F, Tinneberg HR, Eichenlaub-Ritter U. 2005. Non-invasive method to assess genotoxicity of nocodazole interfering with spindle formation in mammalian oocytes. Reprod Toxicol 19:459–471.

Touati SA, Wassmann K. 2016. How oocytes try to get it right: spindle checkpoint control in meiosis. Chromosoma 125:321-335.

Webster A, Schuh M. 2017. Mechanisms of aneuploidy in human eggs. Trends Cell Biol 27:55-68.

Wei Z, Greaney J, Zhou C, Homer H. 2018. Cdk1 inactivation induces post-anaphase-onset spindle migration and membrane protrusion required for extreme asymmetry in mouse oocytes. Nature Comm 9:4029. DOI: 10.1038/s41467-018-06510-9.

Relationship: 723: Altered, Chromosome number leads to Increase, Aneuploid offspring

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Chemical binding to tubulin in oocytes leading to aneuploid offspring	adjacent	High	

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
mouse	Mus musculus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

Sex Applicability

Sex	Evidence

Sex	Evidence
Female	High

This is based on evidence in humans and mice, but is broadly applicable to all eukaryotic species.

Key Event Relationship Description

Development of a conceptus from a gamete containing an abnormal number of chromosomes results in an aneuploid offspring. Whether the aneuploid conceptus results in a viable offspring is dependent on the chromosome involved in the aneuploidy. Viable aneuploidies in humans include chromosomes 13, 18 and 21, and the sex chromosomes.

Evidence Supporting this KER

Strong.

Biological Plausibility

It is well established that in the majority of cases of human offspring with an aneuploid condition, the extra chromosome is inherited from one of the parents. In humans, it is known that aneuploidy occurs more frequently in female germ cells. It has been known for a long time that there is a strong association between increasing maternal age and increasing risk of aneuploid offspring.

Empirical Evidence

Aneuploidy arising during meiosis in germ cells represents the most common chromosomal abnormality at birth and is the leading cause of pregnancy loss in humans. The presence of aneuploid eggs in humans ranges (depending on age), but is approximately 20%. In parallel, approximately 10–30% of human zygotes are aneuploid. 50% of human pregnancies are spontaneously aborted; of these, 50% are due to aneuploidy. Finally, approximately 0.3% of human newborns are aneuploid. These data are summarized in Hassold et al. [2007] and Nagaoka et al. [2012]. It is widely accepted that human oocytes are particularly susceptible to chromosome mis-segregation [Hassold et al., 2007; Hunt and Hassold, 2002; Nagaoka et al., 2012]. Trisomy 21 or Down syndrome, with an occurrence of ~1/720 births, is the most common genetic abnormality in newborns [Hassold et al., 2007]. The etiology of human aneuploidy is still not well understood, although there is strong evidence supporting a preferential occurrence during female meiosis I and a positive correlation with maternal age [Hunt and Hassold, 2002; Nagaoka et al., 2012; Webster and Schuh, 2017].

Uncertainties and Inconsistencies

None.

Quantitative Understanding of the Linkage

There is limited data on the quantitative relationship between aneuploidy in oocytes and aneuploidy in the offspring. It is difficult to compare the frequencies of aneuploid in oocytes with that in offspring because the great majority of aneuploid embryos are eliminated during pregnancy. However, the majority of individuals who are born with aneuploid conditions are constitutionally aneuploid strongly suggesting that this condition was already present at conception. Indeed, experimental data in rodent support a direct relationship. Some of these results deal with chemicals such as griseofulvin [Marchetti et al., 1992; Tiveron et al., 1992] and taxol [Mailhes et al., 1999] that are not included in this AOP because of uncertainty about the MIE (griseofulvin) or because chemical binding results in the stabilization of microtubules rather than depolymerization (taxol). Nevertheless, together with data with colchicine [Maihles et al., 1990], the available data suggest that the frequencies of aneuploidy before and after fertilization are in general agreement with each other. In addition, data with mice deficient in SAC proteins, which have high levels of female germ cell aneuploidy, show little support for selection against aneuploid eggs at fertilization [Leland et al., 2009].

Response-response relationship

As mentioned above, it is difficult to evaluate the response-response relationship between these two KEs because the majority of aneuploid conceptuses are eliminated during pregnancy. There are a few studies that report on the frequency of aneuploidy in oocytes (KEupstream) and the frequency of aneuploidy in zygotes, only a small portion of which will result in an increase in aneuploid offspring (KEdownstream). Studies with colchicine [Mailhes et al., 1990], griseofulvin [Tiveron et al, 1992; Marchetti et al., 1992] and taxol [Mailhes et al., 199] all show that the frequencies of aneuploid oocytes and aneuploid zygotes are similar suggesting a linear relationship at least between these two events.

Time-scale

Chemically induced aneuploidy is occurring around the time of ovulation when the oocyte completes the first meiotic division. Fertilization generally occurs within a few hours from ovulation and thus the generation of the aneuploid conceptus follows the KEupstream by a matter of hours. The KEdownstream, that is aneuploid offspring, is determined by the duration of pregnancy in the species, weeks in the mouse, months in humans, but again, only a small portion of the aneuploid zygotes will result in a live offspring.

Known modulating factors

There are no studies that have looked at whether specific chromosomes are more prone to undergo chemically induced aneuploidy, thus, it can be assumed, that the fraction of zygotes that are aneuploid for chromosomes that are compatible with life will also show a linear relationship as that observed between aneuploid oocytes and zygotes.

Known Feedforward/Feedback loops influencing this KER

There are no known feedbacks loops.

References

Hassold T, Hall H, Hunt P. 2007. The origin of human aneuploidy: Where we have been, where we are going. Hum Mol Genet 16: R203–R208.

Hunt PA, Hassold TJ. 2002. Sex matters in meiosis. Science 296:2181–2183.

Leland S, Nagarajan P, Polyzos A, Thomas S, Samaan G, Donnell R, Marchetti F, Venkatachalam S. 2009. Heterozygosity for a Bub1 mutation causes female-specific germ cell aneuploidy in mice. Proc Natl Acad Sci USA 106:12776-12781.

Mailhes JB, Aardema MJ, Marchetti F. 1990. Cytogenetic analysis of mouse oocytes and one-cell zygotes as a potential assay for heritable germ cell aneuploidy. Mutat Res 242:89-100.

Mailhes JB, Carabatsos MJ, Young D, London SN, Bell M, Albertini DF. 1999. Taxol-induced meiotic maturation delay, spindle defects, and aneuploidy in mouse oocytes and zygotes. Mutat Res 423:79-90.

Marchetti F, C Tiveron, B Bassani and F Pacchierotti. 1992. Griseofulvin-induced aneuploidy and meiotic delay in female mouse germ cells, II. Cytogenetic analysis of one-cell zygotes. Mutat Res 266:151-162.

Nagaoka SI, Hassold TJ, Hunt PA. 2012. Human aneuploidy: Mechanisms and new insights into an age-old problem. Nat Rev Genet 13:493–504.

Tiveron C, F Marchetti, B Bassani and F Pacchierotti. 1992. Griseofulvin-induced aneuploidy and meiotic delay in female mouse germ cells, I. Cytogenetic analysis of metaphase II oocytes. Mutat Res 266:143-150.

Webster A, Schuh M. 2017. Mechanisms of aneuploidy in human eggs. Trends Cell Biol 27:55-68.

List of Non Adjacent Key Event Relationships

Relationship: 724: Binding, Tubulin leads to Altered, Chromosome number

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Chemical binding to tubulin in oocytes leading to aneuploid offspring	non-adjacent	High	

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Mixed	High

Data for this KER are available in vitro and in vivo, and in a variety of mammalian species including humans.

Key Event Relationship Description

In this KER, chemicals that bind to tubulin indirectly lead to altered chromosome numbers. This is because tubulin binding by chemicals interferes with tubulin polymerization leading to microtubule depolymerization, abnormal spindle structure/morphology and subsequent chromosome mis-segregation. The relationship is indirect because there are no studies that have measured all KEs leading up to the AO. However, as described in more details below, there are plenty of studies showing that exposure to spindle poisons induces aneuploidy in female germ cells. This relationship has been shown in vitro and in vivo, and in somatic cells as well as in germ cells.

Evidence Supporting this KER

Strong.

Biological Plausibility

Accurate chromosome segregation requires the temporally regulated and coordinated interaction of many cellular components including protein kinases and phosphatases, topoisomerases, the anaphase-promoting complex (APC), proteasomes, mitotic and meiotic spindle, centrosomes and kinetochores [Orr et al., 2015]. Disruption of any of these processes by chemicals can potentially result in aneuploidy [Parry et al., 2002]. There is extensive knowledge of cellular processes associated with chromosome segregation in both somatic cells [Collin et al., 2013; London et al., 2014; Musacchio, 2015] and germ cells [Polanski, 2013; Touati and Wassmann, 2016; Bennabi et al., 2016]. Although many of these cellular components and processes are shared between somatic cells and germ cells, there are features that are unique to germ cells, in general, and female germ cells specifically [Hunt and Hassold, 2002; Webster and Schuh, 2017].

Unique to germ cells are the processes that take place during the first meiotic division when homologous chromosomes must segregate to opposite poles of the cell. Homologous chromosome segregation is possible because they are paired in bivalents physically attached at chiasmata and the sister kinetochores of each chromosome are held together by complexes of cohesion proteins, behaving as a unique monooriented structure with respect to spindle microtubules [reviewed by Eichenlaub-Ritter, 2012]. This is at variance with the second meiotic division and mitotic division when segregation involves the two sister chromatids of each chromosome. Different mechanisms have been proposed to cause aneuploidy in germ cells, including: (1) nondisjunction of homologous chromosomes; (2) premature separation of homologous chromosomes or sister chromatids; and (3) recombination defects [Nagaoka et al., 2012; Zelazowski et al., 2017]. Each of these mechanisms interacts and contributes to the genesis of aneuploidy through a complex interplay of molecular and cellular events [Nagaoka et al., 2012]. Unique to female germ cells is also the formation of the meiotic spindle in the absence of centrioles, as described before, and the reduced stringency of the SAC that allows progression of meiosis even in the presence of misaligned chromosomes, and the long time that oocytes are arrested at the end of meiotic prophase with possible progressive degradation of cohesion proteins [Hunt and Hassold, 2002; Nagaoka et al., 2012; Webster and Schuh, 2017].

This KER indirectly links chemical binding to tubulin to aneuploidy. A diverse array of chemical agents are well established to induce aneuploidy, with the majority of these agents operating through binding to tubulin to impair spindle function, chromosome dynamics and ultimately segregation [reviewed in Parry et al., 2002; and in Pacchierotti and Eichenlaub-Ritter, 2011]. However, an extensive amount of work in this field has focused on gametes thus, we focus on chemically-induced aneuploidy in germ cells. For a summary of chemically-induced aneuploidy in somatic cells the reader is referred to a few key reviews [e.g., Adler, 1993; Leopardi et al., 1993; Aardema et al., 1998].

There is extensive evidence in mammalian models that chemicals can induce aneuploidy by interfering with the proper functioning of the meiotic spindle and other aspects of chromosome segregation. The aneugenic activity of microtubule disrupting agents was also recently demonstrated using a *Caenorhabditis elegans* screening platform for the rapid assessment of chemical effects on germline function [Allard et al., 2013]. About 20 chemicals have been shown to induce aneuploidy in mammalian oocytes in vivo and the majority of these chemicals are tubulin binders (i.e., they interfere with microtubule dynamics through tubulin binding during meiosis) [Mailhes and Marchetti, 1994, 2005; Pacchierotti and Eichenlaub-Ritter, 2011]. Collectively, these studies suggest that the main window for the induction of aneuploidy in oocytes is restricted to the periovulation period with a peak of sensitivity around the resumption of meiosis and the induction of ovulation. Depending on dose and time, spindle inhibitors can induce aneuploidy in almost 100% of oocytes [reviewed in Mailhes and Marchetti, 2005], suggesting that the disruption of microtubule and spindle dynamics is a very sensitive target for the induction of aneuploidy in female germ cells. Although the majority of the available studies investigated the induction of aneuploidy during meiosis I, there is evidence that the two meiotic divisions have similar sensitivity to chemically-induced aneuploidy [Marchetti et al., 1996].

Empirical Evidence

*The e*ffects of potential aneuploidy-inducing agents on microtubule assembly in vitro has been investigated by Brunner et al. [1991] and Wallin and Hartely-Asp [1993]. These authors explored the potency of 10 chemicals in binding tubulin relative to colchicine, and reported that there is a good correlation between the efficiency of these chemicals to interfere with microtubule assembly and their known aneugenic potential.

The evidence that colchicine and other chemicals that bind to tubulin induce aneuploidy in rodent oocytes is very strong [reviewed in Mailhes and Marchetti, 1994, 2005]. Most of the data with colchicine have been collected in mice [Tease and Fisher, 1986; Mailhes and Yuan, 1987; Mailhes et al., 1988; Mailhes et al., 1990], but evidence for colchicine-induced aneuploidy is also available in Chinese hamster [Sugawara and Mikamo, 1980] and Djungarian hamster [Hummler and Hansmann, 1985] oocytes

(Supplementary Table 1). Specifically, intraperitoneal injection of 0.25 mg/kg bw colchicine before the onset of the first meiotic spindle formation increases the frequency of hyperhaploid metaphase II oocytes from 0.4 to 11.2% in mice [Tease and Fisher, 1986]. In addition, the percentage of hyperhaploid mouse oocytes induced by 0.2 mg/kg bw colchicine varies as a function of injection time [Mailhes and Yuan, 1987], with a maximum effect (10-fold increase over control level) at the time of spindle assembly (around 12 hr before ovulation). However, statistically significant effects were also reported up to 4 hr before and after this period of maximum sensitivity.

In Chinese hamsters, a single intraperitoneal injection of 3 mg/kg bw colchicine induced a 10-fold increase of hyperhaploid oocytes (from 0.8 in the control to 8.6% in the exposed group) [Sugawara and Mikamo, 1980]. Similarly, a statistically significant increase from 3.5 to 11.7% hyperhaploid oocytes was observed in Djungarian hamsters [Hummler and Hansmann, 1985]. Interestingly, the experiment with Chinese hamsters was carried out under natural ovulation conditions, i.e. without the use of exogenous hormones applied in all other studies to synchronize the oestrus cycle and increase the number of oocytes ovulated by each female. This provides experimental evidence for the lack of influence of superovulation on the aneugenic effects of colchicine. It should be noted that the same levels of meiotic arrest and aneuploidy were induced in hamsters by a dose 10 times higher than in mice. Indeed, Midgley et al. showed that hamster cells were more resistant to colchicine than mouse and human cells [Midgley et al., 1959]. While these results suggest that species-specific differences may exist in the sensitivity of oocytes to the aneugenic effects of colchicine, the consistent positive findings across species provide strong evidence to support the causal relationship between colchicine binding to tubulin and induction of aneuploidy.

Studies by Mailhes and coworkers in mice describe a dose-effect relationship for the induction of aneuploid oocytes by intraperitoneal injection of colchicine between 0.1 and 0.4 mg/kg bw [Mailhes et al., 1988; Mailhes et al., 1990], demonstrating that as the incidence of tubulin binding by colchine increases, so does the incidence of aneuploidy. Doses of 0.5 mg/kg bw and higher resulted in the arrest of all mouse oocytes at the metaphase I stage. The results for 0.2 mg/kg bw confirmed the 10-fold increase over controls reported in a previous study [Mailhes and Yuan, 1987]. At 0.3 and 0.4 mg/kg bw, the percentages of hyperhaploid oocytes increased, reaching 20.8 and 23.5%, respectively. Interestingly, no significant increase was caused by 0.1 mg/kg bw colchicine, suggesting that a threshold exists for colchicine-induced aneuploidy in mouse oocytes. The presence of a threshold for the induction of aneuploidy by tubulin binders is broadly accepted in somatic cells as well [Cammerer et al., 2010; Elhajouji et al., 2011].

The hypothesis that a certain level of tubulin damage is needed to impair spindle function is in agreement with the observation that at higher doses the “severity” of the aneugenic effect increases, with more oocytes containing not one but several supernumerary chromosomes [Mailhes et al., 1988; Mailhes et al., 1990]. Mailhes and coworkers also compared the dose-effect relationships for oocyte aneuploidy induction after intraperitoneal and oral colchicine administration [Mailhes et al., 1990]. Not surprisingly, due to the reduced bioavailability of the compound using this route, they showed that ten times higher oral doses of colchicine are needed to induce about the same level of effect induced by intraperitoneal injection. Also after oral treatment, there is a No Observed Effect Dose, corresponding, in this case, to 1 mg/kg bw. An effectiveness ratio of 10 between the two administration routes also occurs for the induction of metaphase I blocked oocytes.

Overall, about 20 chemicals have been shown to induce aneuploidy in mammalian oocytes. The majority of these chemicals are spindle poisons that are known to bind to microtubules and interact with tubulin in a manner analogous to colchicine. Data on the induction of aneuploidy in oocytes after exposure to these chemicals is reviewed in Mailhes and Marchetti [1994, 2005] and Marchetti et al. [2016].

Uncertainties and Inconsistencies

We are not aware of any chemical that bind to tubulin and does not cause aneuploidy, providing that a high enough dose/concentration was tested.

Quantitative Understanding of the Linkage

Although quantitative models have not been developed, the qualitative relationship is described above. In vivo studies indicate that timing prior to meiotic division must be carefully considered because of the different half lives of the chemicals bound to tubulin. Depending on dose and time, chemicals that bind to tubulin can induce aneuploidy in almost 100% of oocytes [Mailhes and Marchetti, 2005], suggesting that the disruption of microtubule and spindle dynamics is a very sensitive target for the induction of aneuploidy in female germ cells.

Response-response relationship

It is difficult to compare the response-response relationship between these two KEs, as binding to tubuline (KEupstream) is generally measured in an acellular system or in vitro, while altered chromosome nubmer (KEdownstream) is measured in vivo. However, Brunner et al. [1991] and Wallin and Hartely-Asp [1993] analyzing the ability of 10 chemicals to interfere with microtubule assembly reported that there is a good correlation between the efficiency of microtubule assembly interference and the anuegenic potential of each chemical. That is, chemicals that interfered with microtubule assembly at low concentrations are strong aneugens (eg, colchicine, vinblastine); while chemicals that did not affect the steady state of microtubule assembly do not induced aneuploidy or are very weak inducers (eg, diazepam, cadmium chloride).

Time-scale

Binding to tubulin is occurring on the time scale seconds (acellular systems) and minutes (in vitro). In vivo, the time-scale is determined by the route of administration and the ADME characteristics of the chemical. For the induction of aneuploidy, chemical

binding to tubulin must occur within a short time range before the completion of the first meiotic division. Mailhes and Yuan [1987] showed that the induction of aneuploid oocytes following exposure to colchicine is maximum when administered 12 hr before ovulation and is reduced when given more or less than 12 hours.

Known modulating factors

As described above, time of exposure with respect to ovulation is a modulating factor.

Known Feedforward/Feedback loops influencing this KER

No known feedback loops.

References

Aardema MJ, Albertini S, Arni P, Henderson LM, Kirsch-Volders M, Mackay JM, Sarrif AM, Stringer DA, Taalman RD. 1998. Aneuploidy: a report of an ECETOC task force. Mutat Res 410:3-79.

Adler ID. 1993. Synopsis of the in vivo results obtained with the 10 known or suspected aneugens tested in teh CEC collaborative study. Mutat Res 287:131-137.

Allard P, Kleinstreuer NC, Knudsen TB, Colaiacovo MP. 2013. A C. elegans screening platform for the rapid assessment of chemical disruption of germline function. Environ Health Perspect 121:717–724.

Bennabi I, Terret ME, Verlhac MH. 2016. Meiotic spindle assembly and chromosome segregation in oocytes. J Cell Biol 215:611-619.

Brunner M, Albertini S, Würgler FE. 1991. Effects of 10 known or suspected spindle poisons in the in vitro porcine brain tubulin assembly assay. Mutagen 6:65-70.

Cammerer Z, Schumacher MM, Kirsch-Volders M, Suter W, Elhajouji A. 2010. Flow cytometry peripheral blood micronucleus test in vivo: determination of potential threshold for aneuploidy induced by spindle poisons. Environ Mol Mutagen 51:278-284.

Collin P, Nashchekina O, Walker R, Pines J. 2013. The spindle assembly checkpoint works like a rheostat rather than a toggle switch. Nat Cell Biol 15:1378–1385.

Eichenlaub-Ritter U. 2012. Female meiosis and beyond: more questions than answers? Reprod Biomed Online 24:589-590.

Elhajouji A, Lukamowicz M, Cammerer Z, Kirsch-Volders M. 2011. Potential thresholds for genotoxic effects by micronucleus scoring. Mutagenesis 26:199-204.

Hassold T, Hall H, Hunt P. 2007. The origin of human aneuploidy: Where we have been, where we are going. Hum Mol Genet 16: R203–R208.

Hummler E, Hansmann I. 1985. Preferential nondisjunction of specific bivalents in oocytes from Djungarian hamsters (Phodopus sungorus) following colchicine treatment. Cytogenet Cell Genet 39:161–167.

Hunt PA, Hassold TJ. 2002. Sex matters in meiosis. Science 296:2181–2183.

Leopardi P, Zijno A, Bassani B, Pacchierotti F. 1993. In vivo studies on chemically induced aneuploidy in mouse somatic and germinal cells. Mutat Res 287:119-130.

London N, Biggins S. 2014. Signalling dynamics in the spindle checkpoint response. Nat Rev Mol Cell Biol 15:736–747.

Mailhes JB, Marchetti F. 1994. The influence of postovulatory ageing on the retardation of mouse oocyte maturation and chromosome segregation induced by vinblastine. Mutagenesis 9:541–545.

Mailhes JB, Marchetti F. 2005. Mechanisms and chemical induction of aneuploidy in rodent germ cells. Cytogenet Genome Res 111: 384–391.

Mailhes JB, Yuan ZP. 1987. Differential sensitivity of mouse oocytes to colchicine-induced aneuploidy. Environ Mol Mutagen 10:183–188.

Mailhes JB, Preston RJ, Yuan ZP, Payne HS. 1988. Analysis of mouse metaphase II oocytes as an assay for chemically induced aneuploidy. Mutat Res 198:145–152.

Mailhes JB, Yuan ZP, Aardema MJ. 1990. Cytogenetic analysis of mouse oocytes and one-cell zygotes as a potential assay for heritable germ cell aneuploidy. Mutat Res 242:89–100.

Marchetti F, Mailhes JB, Bairnsfather L, Nandy I, London SN. 1996. Dose-response study and threshold estimation of griseofulvininduced aneuploidy during female mouse meiosis I and II. Mutagenesis 11:195–200.

Midgley AR, Pierce B, Dixon FJ. 1959. Nature of colchicine resistance in golden hamster. Science 130:40–41.

Musacchio A. 2015. The molecular biology of spindle assembly checkpoint signaling dynamics. Curr Biol 25:R1002-R1018.

Nagaoka SI, Hodges CA, Albertini DF, Hunt PA. 2011. Oocyte-specific differences in cell-cycle control create an innate susceptibility to meiotic errors. Curr Biol 21:651-657.

Nagaoka SI, Hassold TJ, Hunt PA. 2012. Human aneuploidy: Mechanisms and new insights into an age-old problem. Nat Rev Genet 13:493–504.

Orr B, Godek KM, Compton D. 2015. Aneuploidy. Curr Biol 25:R523-548.

Pacchierotti F, Eichenlaub-Ritter U. 2011. Environmental hazard in the aetiology of somatic and germ cell aneuploidy. Cytogenet Genome Res 133:254-268.

Parry EM, Parry JM, Corso C, Doherty A, Haddad F, Hermine TF, Johnson G, Kayani M, Quick E, Warr T, Williamson J. 2002. Detection and characterization of mechanisms of action of aneugenic chemicals. Mutagenesis 17:509-21.

Polanski Z. 2013. Spindle assembly checkpoint regulation of chromosome segregation in mammalian oocytes. Reprod Fertil Dev 25:472-483

Sugawara S, Mikamo K. 1980. An experimental approach to the analysis of mechanisms of meiotic nondisjunction and anaphase lagging in primary oocytes. Cytogenet Cell Genet 28:251-264.

Tease C, Fisher G. 1986. Oocytes from young and old female mice respond differently to colchicine. Mutat Res 173:31–34.

Touati SA, Wassmann K. 2016. How oocytes try to get it right: spindle checkpoint control in meiosis. Chromosoma 125:321-335.

Wallin M, Hartley-Asp B. 1993. Effects of potential aneuploidy inducing agents on microtubule assembly in vitro. Mutat Res 287:17-22.

Webster A, Schuh M. 2017. Mechanisms of aneuploidy in human eggs. Trends Cell Biol 27:55-68.

Zelazowski MJ, Sandoval M, Paniker L, Hamilton HM, Han J, Gribbell MA, Kang R, Cole F. 2017. Age-Dependent Alterations in Meiotic Recombination Cause Chromosome Segregation Errors in Spermatocytes. Cell 171:601-614