12.07 Epigenetic Transgenerational Toxicology

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Abbreviations

BPA bisphenol A

DES diethylstilbesterol

PGC primordial germ cell

12.07.1 Introduction

The majority of environmental factors and toxicants do not have the ability to alter DNA sequence or promote genetic mutations (Jirtle and Skinner 2007; Szyf 2007). This is due in large part to the stability of the genome. However, most will promote abnormal phenotypes or disease. Early life exposures often lead to later life adult onset disease (Hanson and Gluckman 2008). This toxicology is not mediated through traditional genetic mechanisms, but instead through altered molecular processes such as epigenetics (Jirtle and Skinner 2007; Morgan and Whitelaw 2009; Waterland 2009). Therefore, in the event these environmental factors promote a heritable or familial transmission of the disease phenotype, it often involves non-Mendelian inheritance.

The heritable transmission of toxicological phenotypes is referred to as transgenerational inheritance (Jirtle and Skinner 2007; Whitelaw and Whitelaw 2008). The vast majority of environmental exposures will involve somatic cells and, as such, will not promote a transgenerational phenotype, but still may be critical for the individual exposed in regard to potential adult onset disease (Jirtle and Skinner 2007). In the event a germ-line toxicology is involved, then the exposure has the potential to promote a transgenerational phenotype (**Table 1**). Therefore, transgenerational toxicology can be considered as a subset of toxicology involving direct actions of environmental factors or toxicants on the germ line.

Toxicology studies often involve a correlation between exposure and the development of an abnormal phenotype or disease. The future of the field of toxicology lies in elucidation of the molecular and cellular mechanisms involved in the actions of the environmental factor or toxicant. A basic understanding of the molecular mechanisms involved in toxicology will dramatically facilitate risk assessment and provide diagnostic tools for exposure analysis and for developing potential treatments for exposures. Although susceptibility to exposure and genetics are important molecular factors, an alternate mechanism such as the role of epigenetics is critical to consider in future toxicology research.

12.07.2 Transgenerational Phenotypes

As discussed, the majority of toxicology involves direct exposures and involves somatic tissues. In contrast, transgenerational phenotypes and toxicology exclude direct exposure and must be transmitted through multiple generations (Jirtle and Skinner 2007; Skinner 2008). For example, exposure of a gestating female provides direct exposure of the F0 generation female, the F1 generation embryo, and the germ line that will generate the F2 generation (Skinner 2008). Therefore, a phenotype in the F3 generation that extends from the exposure history has an obligate transgenerational toxicology. The toxicology observed in the F0 and F1 generations,

Table 1 Sites of action and phenotypes of environmental factors and toxicants

Site of action	*Biological response and toxicology*
Somatic cells	Allows tissue-specific toxicology and critical for adult onset disease in the individual exposed, but not capable of transmitting a transgenerational phenotype
Germ cells	Allows transmission between generations, and in the absence of direct exposure promotes a transgenerational phenotype

Table 2 Transgenerational versus multigenerational phenotypes and toxicology

Phenotype	*Exposure*	*Definition*
Multigenerational	Direct	Coincident direct exposure of multiple generations to an environmental factor or toxicant promoting a toxicology in the multiple generations exposed
Transgenerational	None, except the initial generation	After the initial exposure, the transgenerational phenotype is transmitted through the germ line in the absence of direct exposure

as well as in the F2 generation germ line, was due to direct exposures (Jirtle and Skinner 2007; Skinner 2008). Importantly, the ability of a direct exposure to influence multiple generations is defined as a multiple generation phenotype (**Table 2**) rather than a transgenerational phenotype. A transgenerational phenotype requires the absence of a direct exposure and toxicology (**Table 2**).

One class of environmental compounds or toxicants involved in such phenotypes are endocrine disruptors that interfere with normal hormone signaling. A classic example of a multigenerational toxicology involves the pharmaceutical agent with estrogen agonist activity, namely diethylstilbesterol (DES) (Newbold 2008). Exposure of a gestating female to DES was found to promote an abnormal reproductive tract and gonadal dysfunction in the F1 generation males and females, as well as abnormal female reproductive tract function in the F2 generation (Brouwers *et al.* 2006; Newbold *et al.* 2006). Interestingly, the phenotypes of the F1 and F2 generations differ. Limited information exists on the F3 generation in humans, but a major phenotype was not observed in F3 generation rodent models (Brouwers *et al.* 2006; Newbold 2008; Newbold *et al.* 2006). It is possible that DES promotes a transgenerational phenotype, but extended generations need to be investigated (Newbold *et al.* 2006). Another example of a multigenerational toxicology is a study with flutamide (Anway *et al.* 2008a). This antiandrogenic endocrine disruptor after exposure of a gestating female promoted abnormality in the testis of F1 generation and affected skeletal development in F2 generation, but had no effects on F3 generation (Anway *et al.* 2008). Again the F1 and F2 generation phenotypes were distinct. In contrast, another endocrine disruptor vinclozolin did promote a transgenerational phenotype in the F3 generation (Anway *et al.* 2008). There are many environmental factors and toxicants that promote a toxicology for multiple generations involving direct exposure of the different generations (Hanson and Gluckman 2008; Jirtle and Skinner 2007). These multigenerational exposures and phenotypes are not transgenerational phenotypes, although they are critical in assessing the toxicology of an environmental agent.

Transgenerational phenotypes and toxicology require the germ line for transmission between generations. These transgenerational phenotypes occur in the absence of direct exposure (**Table 2**). Somatic cell targets are critical and common in toxicology to promote adult onset disease and phenotypes, but are not able to transmit the phenotype transgenerationally without continued direct exposure. Therefore, the critical target cell for transgenerational phenotypes and toxicology is the germ line.

12.07.3 Epigenetics

Epigenetics is defined as molecular factors and processes around DNA that regulate genome activity independent of DNA sequence. Regulatory processes such as DNA methylation, histone modifications, chromatin structure, and noncoding RNA are all examples of epigenetic factors. Epigenetic regulation of genome activity is critical in the development and maintenance of cellular function and differentiation.

Table 3 Examples of epigenetic processes

DNA methylations	Methylcytosine at CpG sites
Histone modifications	Methylation and acetylation at lysine residues
Chromatin structure	Loop and bend structures and nuclear matrix associations
Noncoding RNA	Small RNA influencing RNA stability and gene expression

The term epigenetics was originally coined by Conrad Waddington (1940) in his discussion of gene–environment interactions. The first molecular factor identified was DNA methylation in the 1970s (Holliday and Pugh 1975) followed by histone modifications in the 1990s (Jirtle and Skinner 2007). Therefore, the majority of molecular elements of epigenetic regulatory processes have only been recently elucidated (Jirtle and Skinner 2007). Epigenetic processes are known to involve a number of multiple molecular processes including DNA methylation, histone modifications, chromatin structural change, and noncoding RNAs (**Table 3**). These processes do not directly involve DNA sequence and hence are considered epigenetic and are equally likely important in regulating genome activity (i.e., gene expression) as the primary DNA sequence (i.e., genetics).

The ability of environmental factors or toxicants to influence epigenetic processes associated with altered phenotypes has been demonstrated in different model systems. One of the first observations of how the environment could influence epigenetics and phenotype was made in plants (Cuzin *et al.* 2008). A classic mouse model is the agouti model (Dolinoy 2008; Rakyan *et al.* 2003). An epigenetic modification of the agouti locus alters coat color and can lead to metabolic disease (Dolinoy 2008; Waterland 2009). Environmental factors such as nutrition and toxicants such as the estrogenic substance bisphenol A (BPA), a common plasticizer to which humans are exposed, can modulate the DNA methylation state in the murine agouti locus to alter phenotypes (Dolinoy *et al.* 2007). There are other environmental factors and model systems that promote phenotypic changes through epigenetic processes (Hanson and Gluckman 2008; Jirtle and Skinner 2007; Morgan and Whitelaw 2009).

As discussed, the majority of toxicology and actions of environmental factors are on somatic cells (**Table 2**). Examples of specific toxicants acting on a given tissue to promote a disease state in many cases involve epigenetic processes. An example is the action of BPA on the pubertal prostate to promote an epigenetic effect on DNA methylation that is associated with adult onset prostate disease (Ho *et al.* 2006). Similar effects on prostate disease have also been observed with the fungicide vinclozolin (Cowin *et al.* 2008). The majority of environmental factors influencing adult onset disease will likely involve direct or indirect epigenetic modifications in the tissue or somatic cells involved (Jirtle and Skinner 2007). These somatic cell effects will be critical in the etiology of disease in the individual exposed, but the toxicology will not become transgenerational without continued exposure of subsequent generations.

Since the germ line is required for the transmission of genetic influence between generations, in the event a permanent epigenetic modification of the germ line occurred, an epigenetic transgenerational phenomenon could occur. In considering germ-line biology there are critical periods of development where epigenetic modifications could be influenced. During embryonic development in mammalian species, the primordial germ cells (PGCs) migrate to the developing gonad prior to sex determination as a pluripotent stem cell (Allegrucci *et al.* 2005; Durcova-Hills *et al.* 2006; Trasler 1998). Upon gonadal sex determination, the germ cell develops into a male or a female germ line during the initial stages of sex determination. The female germ line develops and then enters meiosis in the developing embryonic ovary (Allegrucci *et al.* 2005; Durcova-Hills *et al.* 2006; Trasler 1998). The male germ line continues to proliferate until immediately prior to birth and then resumes proliferation after birth until puberty. In the adult, the female germ line undergoes oogenesis during follicle development to generate oocytes. The male germ line develops from spermatogonial stem cells and undergoes spermatogenesis for the production of spermatozoa in the testis.

Epigenetic programming of the germ line occurs during the migration of the PGCs in the embryo. The migrating PGCs undergo an erasure, or 'demethylation' of the DNA during migration and colonize the early bipotential gonad prior to gonadal

sex determination (Allegrucci *et al.* 2005; Durcova-Hills *et al.* 2006; Trasler 1998). Then, when gonadal sex determination is initiated, the PGCs develop female or male germ cell lineage and 'remethylate' the DNA in a male- or female-specific manner. Therefore, the germ-cell DNA is demethylated and remethylated during gonadal sex determination and is potentially sensitive to environmental factors (Allegrucci *et al.* 2005; Durcova-Hills *et al.* 2006; Trasler 1998).

Although there are alterations in the male and female germ-line epigenomes (i.e., DNA methylation) during gametogenesis in the adult gonads (Zamudio *et al.* 2008), the embryonic period of gonadal sex determination is the most sensitive to environmental insults that can potentially influence the epigenome. In contrast to the female germ cell that could allow the transmission of numerous types of epigenetic processes, the male germ cell, during spermatogenesis, replaces the majority of histones with protamines, involves DNA condensation to eliminate chromatin structure, and then silences the genome for expression of noncoding RNAs (Godmann *et al.* 2009). Therefore, the primary epigenetic process that is transmitted through the male germ line is DNA methylation. An example of this involves imprinted genes that have a specific pattern of DNA methylation transmitted through a parent of origin allele to subsequent progeny (Ideraabdullah *et al.* 2008). These imprinted sites regulate gene expression in an allele-specific manner. Therefore, DNA methylation has been established as a transgenerational epigenetic mechanism (Ideraabdullah *et al.* 2008). Due to the reprogramming of the germ line, gonadal sex determination is one of the most sensitive periods for environmental exposures to influence the germ line and create a transgenerational phenotype.

12.07.4 Epigenetic Transgenerational Phenomena

The initial observations of epigenetic transgenerational phenotypes were made in plants and involved DNA methylation and paramutation mechanisms (Cuzin *et al.* 2008; Mathieu *et al.* 2007). The observation was made in mammals when transient exposure to vinclozolin during gonadal sex determination was found to promote an adult onset disease in the F1 generation as well as in subsequent generations (i.e., F1–F4) (Anway *et al.* 2005). This was partly attributed to DNA methylation changes in the male germ line (i.e., sperm) and promoted transgenerational changes in the transcripts for a number of tissues (Anway and Skinner 2008; Anway *et al.* 2008; Skinner *et al.* 2008). The adult onset diseases observed included testis abnormalities, prostate disease, kidney disease, immune abnormalities, and tumor development (Anway *et al.* 2006). Therefore, environmental exposure of a gestating female to vinclozolin during the critical period of gonadal sex determination appears to have modified the male sperm epigenome to allow the transgenerational transmission of adult onset disease (Anway *et al.* 2005; Jirtle and Skinner 2007). A recent study suggests that the transgenerational actions of vinclozolin may not involve its antiandrogen endocrine disruptor actions, but rather other unknown actions (Anway *et al.* 2008). These studies provide one of the first examples of an epigenetic transgenerational phenomenon (Anway *et al.* 2005).

Several other environmental factors and models are now being used to describe further these epigenetic transgenerational phenomena (Katz *et al.* 2009; Xing *et al.* 2007). The agouti mouse model was used to document a transgenerational adult onset obesity phenotype (Waterland *et al.* 2008). Modification of the DNA methylation pattern of the agouti locus with environmental toxicants such as BPA modified this phenotype (Dolinoy *et al.* 2007). Another example involves the ability of BPA to promote testis abnormalities for three (F1–F3) generations (Salian *et al.* 2009). Nutritional defects can also promote a transgenerational response (Kaati *et al.* 2007) and influence transgenerational genetic defects (Arai *et al.* 2009). A number of heritable disease states also appear to be of epigenetic origin (e.g., multiple sclerosis) (Chao *et al.* 2009). Therefore, further analysis of the actions of environmental factors and toxicants when exposure could affect the germ line needs to be performed to elucidate the extent epigenetic transgenerational phenomena are involved in adult onset disease and environmental toxicology.

12.07.5 Summary and Future Directions

Epigenetic transgenerational toxicology will require the involvement of the germ line to allow the transmission of an epigenetic abnormality between multiple generations. The ability of environmental factors or toxicants to promote an alteration in the epigenome will be common for somatic tissues, but

less common for the germ line due to the limited developmental period the germ line is sensitive to reprogramming. In the event an altered germ-line epigenome becomes permanently programmed, an epigenetic transgenerational phenotype would be possible. The phenomenon of the fetal basis of adult onset disease has been established (Hanson and Gluckman 2008; Jirtle and Skinner 2007) and epigenetics will likely play a critical role in this process. In consideration of the toxicology of an environmental agent, transient early life exposure in the individual exposed, or transgenerationally if the germ line is involved, is now considered as a causal factor for adult onset disease. Further investigation into the role of epigenetics in disease etiology is now needed to determine if early life toxicology may be a significant factor in disease. Elucidation of the epigenetics involved in transgenerational toxicology would provide insights into the diagnosis of exposure and potential therapeutic targets for disease. Although the prevalence of epigenetic transgenerational toxicology needs to be assessed in various disease states, the role of epigenetics will likely be a major factor to consider in toxicology and medicine in the future.

References

Allegrucci, C.; Thurston, A.; Lucas, E.; Young, L. *Reproduction* **2005**, *129* (2), 137–149.

Anway, M. D.; Cupp, A. S.; Uzumcu, M.; Skinner, M. K. *Science* **2005**, *308* (5727), 1466–1469.

Anway, M. D.; Leathers, C.; Skinner, M. K. *Endocrinology* **2006**, *147* (12), 5515–5523.

Anway, M. D.; Rekow, S. S.; Skinner, M. K. *Reprod. Toxicol.* **2008a**, *26* (2), 100–106.

Anway, M. D.; Rekow, S. S.; Skinner, M. K. *Genomics* **2008b**, *91* (1), 30–40.

Anway, M. D.; Skinner, M. K. *Prostate* **2008**, *68* (5), 517–529.

Arai, J. A.; Li, S.; Hartley, D. M.; Feig, L. A. *J. Neurosci. Methods* **2009**, *29* (5), 1496–1502.

Brouwers, M. M.; Feitz, W. F.; Roelofs, L. A.; Kiemeney, L. A.; de Gier, R. P.; Roeleveld, N. *Hum. Reprod.* **2006**, *21* (3), 666–669.

Chao, M. J.; Ramagopalan, S. V.; Herrera, B. M.; Lincoln, M. R.; Dyment, D. A.; Sadovnick, A. D.; Ebers, G. C. *Hum. Mol. Genet.* **2009**, *18* (2), 261–266.

Cowin, P. A.; Foster, P.; Pedersen, J.; Hedwards, S.; McPherson, S. J.; Risbridger, G. P. *Environ. Health Perspect.* **2008**, *116* (7), 923–929.

Cuzin, F.; Grandjean, V.; Rassoulzadegan, M. *Curr. Opin. Genet. Dev.* **2008**, *18* (2), 193–196.

Dolinoy, D. C. *Nutr. Rev.* **2008**, *66* (Suppl. 1), S7–S11.

Dolinoy, D. C.; Huang, D.; Jirtle, R. L. *Proc. Natl. Acad. Sci. USA* **2007**, *104* (32), 13056–13061.

Durcova-Hills, G.; Hajkova, P.; Sullivan, S.; Barton, S.; Surani, M. A.; McLaren, A. *Proc. Natl. Acad. Sci. USA* **2006**, *103* (30), 11184–11188.

Godmann, M.; Lambrot, R.; Kimmins, S. *Microsc. Res. Tech.* **2009**, *72*, 603–619.

Hanson, M. A.; Gluckman, P. D. *Basic Clin. Pharmacol. Toxicol.* **2008**, *102* (2), 90–93.

Ho, S. M.; Tang, W. Y.; Belmonte de Frausto, J.; Prins, G. S. *Cancer Res.* **2006**, *66* (11), 5624–5632.

Holliday, R.; Pugh, J. E. *Science* **1975**, *187* (4173), 226–232.

Ideraabdullah, F. Y.; Vigneau, S.; Bartolomei, M. S. *Mutat. Res.* **2008**, *647* (1–2), 77–85.

Jirtle, R. L.; Skinner, M. K. *Nat. Rev. Genet.* **2007**, *8* (4), 253–262.

Kaati, G.; Bygren, L. O.; Pembrey, M.; Sjostrom, M. *Eur. J. Hum. Genet.* **2007**, *15* (7), 784–790.

Katz, D. J.; Edwards, T. M.; Reinke, V.; Kelly, W. G. *Cell* **2009**, *137* (2), 308–320.

Mathieu, O.; Reinders, J.; Caikovski, M.; Smathajitt, C.; Paszkowski, J. *Cell* **2007**, *130* (5), 851–862.

Morgan, D. K.; Whitelaw, E. *Nestle Nutr. Workshop Ser. Pediatr. Program* **2009**, *63*, 109–117; discussion 117–119, 259–268.

Newbold, R. R. *Fertil. Steril.* **2008**, *89* (2 Suppl.), e55–e56.

Newbold, R. R.; Padilla-Banks, E.; Jefferson, W. N. *Endocrinology* **2006**, *147* (6 Suppl.), S11–S17.

Rakyan, V. K.; Chong, S.; Champ, M. E.; Cuthbert, P. C.; Morgan, H. D.; Luu, K. V.; Whitelaw, E. *Proc. Natl. Acad. Sci. USA* **2003**, *100* (5), 2538–2543.

Salian, S.; Doshi, T.; Vanage, G. *Life Sci.* **2009**, *85*, 11–18.

Skinner, M. K. *Reprod. Toxicol.* **2008**, *25* (1), 2–6.

Skinner, M. K.; Anway, M. D.; Savenkova, M. I.; Gore, A. C.; Crews, D. *PLoS ONE* **2008**, *3* (11), e3745.

Szyf, M. *Toxicol. Sci.* **2007**, *100* (1), 7–23.

Trasler, J. M. *Semin. Cell Dev. Biol.* **1998**, *9* (4), 467–474.

Waddington, C. H. *Organisers and Genes*; Cambridge Univ. Press: Cambridge, UK, 1940.

Waterland, R. A. *Horm. Res.* **2009**, *71* (Suppl. 1), 13–16.

Waterland, R. A.; Travisano, M.; Tahiliani, K. G.; Rached, M. T.; Mirza, S. *Int. J. Obes. (Lond.)* **2008**, *32* (9), 1373–1379.

Whitelaw, N. C.; Whitelaw, E. *Curr. Opin. Genet. Dev.* **2008**, *18* (3), 273–279.

Xing, Y.; Shi, S.; Le, L.; Lee, C. A.; Silver-Morse, L.; Li, W. X. *PLoS Genet.* **2007**, *3* (9), 1598–1606.

Zamudio, N. M.; Chong, S.; O'Bryan, M. K. *Reproduction* **2008**, *136* (2), 131–146.