Symposium: Nuclear reprogramming and the control of differentiation in mammalian embryos

Epigenetic programming of the germ line: effects of endocrine disruptors on the development of transgenerational disease

Dr Michael Skinner is professor in the School of Molecular Biosciences at Washington State University, USA. He graduated in chemistry at Reed College, Portland and attained his PhD in biochemistry at Washington State University. His post-doctoral fellowship was at CH Best Institute, University of Toronto. Dr Skinner's research is focused on the investigation of how different cell types in a tissue interact and communicate to regulate cellular growth and differentiation, with emphasis in the area of reproductive biology. He is Director of the Washington State University and University of Idaho Center for Reproductive Biology and of the Center for Integrated Biotechnology.

Dr Michael Skinner

Matthew D Anway, Michael K Skinner[1]
Centre for Reproductive Biology, School of Molecular Biosciences, Washington State University, Pullman, WA 99164–4231, USA
[1]Correspondence: Tel: +1 509 3351524; Fax: +1 509 3352176; e-mail: skinner@mail.wsu.edu

Abstract

Epigenetic programming of the germ line occurs during embryonic development in a sex-specific manner. The male germ line becomes imprinted following sex determination. Environmental influences can alter this epigenetic programming and affect not only the developing offspring, but also potentially subsequent generations. Exposure to an endocrine disruptor (i.e. vinclozolin) during embryonic gonadal sex determination can alter the male germ-line epigenetics (e.g. DNA methylation). The epigenetic mechanism involves the alteration of DNA methylation in the germ line that appears to transmit transgenerational adult onset disease, including spermatogenic defects, prostate disease, kidney disease and cancer.

Keywords: *adult onset disease, endocrine disruption, epigenetics, germ line programming, transgenerational*

Introduction

Genomic DNA is the essential building block of all species and is not readily mutated or modified. Epigenetics can be a heritable change in gene expression within the genome that does not directly involve changes to the genomic DNA sequence. Epigenetic regulation of the genome involves factors such as histone modifications (i.e. acetylation and methylation) and DNA methylation that directs chromatin structure and gene transcription. Epigenetic alterations are associated with many human diseases such as cancers (Feinberg, 2004; Schulz and Hatina, 2006), autism (Muhle *et al*., 2004) and Angelman and Beckwith–Wiedemann syndromes (Jiang *et al*., 2004). The epigenetic programming of the germ line appears similar in humans and other mammalian species (Beaujean *et al*., 2004; Fulka *et al*., 2004), such that alterations in germ-line programming may influence genome activity and disease (Steele *et al*., 2005; Tarozzi *et al*., 2007; Yang *et al*., 2007). These non-genomic epigenetic factors are currently speculated to have an important impact on disease risk and transgenerational inheritance (Gluckman *et al*., 2007).

The DNA methylation pattern of the genome becomes reprogrammed following de-methylation and re-methylation processes after fertilization and during early embryonic development. This epigenetic reprogramming during early embryonic cell differentiation transmits a unique DNA methylation pattern to developing organs in the offspring. An additional epigenetic reprogramming event (i.e. DNA methylation) occurs later in development in the germ line during sex determination. A small subset of imprinted genes is transmitted to subsequent generations through the male or female germ line. Imprinted genes have an allele specific DNA methylation pattern and expression that is maternally or

© 2008 Published by Reproductive Healthcare Ltd, Duck End Farm, Dry Drayton, Cambridge CB3 8DB, UK

paternally transmitted between generations. Clearly a number of different epigenetic mechanisms (e.g. histone modifications, chromatin structure and DNA methylation) will be involved in programming the germ line. Alterations in the epigenetic reprogramming of the germ line can promote heritable changes on transcription and disease.

Prior to sex determination during embryonic development the primordial germ cells migrate down the genital ridge and colonize the indifferent biopotential gonad (Hughes, 2001; Kanai *et al*., 2005). As the primordial germ cells migrate down the genital ridge their genomic DNA becomes de-methylated such that the genome prior to and during sex determination is not methylated (Yamazaki *et al*., 2003). Following sex determination, the germ cell DNA is re-methylated in a sex-specific manner (Li *et al*., 2004). In the male, somatic cells in the developing gonad are required for normal germ-cell development and DNA methylation (Hisano *et al*., 2003; Nishino *et al*., 2004). Modification of the methylation pattern of previously identified imprinted genes has been shown to induce disease states (Robertson, 2005). Therefore, alterations in the DNA methylation pattern following sex determination could lead to an epigenetic transgenerational disease state.

Many environmental factors and toxicants have been shown not to directly modify the genomic DNA sequence; however, these factors can cause changes in histone modification or DNA methylation, and this impacts chromatin structure and gene transcription. A consideration of environment–genome interactions requires that epigenetic regulation be considered as one of the components of the molecular basis upon which the environmental factors interact with the genome and result in disease (Herceg, 2007; Weidman *et al*., 2007).

Environmental toxicants have been found to promote transgenerational disease phenotypes (Anway and Skinner, 2006). The transgenerational phenotype has been induced by the endocrine disruptor vinclozolin, an anti-androgenic compound used as a fungicide in the fruit industry (e.g. wineries) (Kelce *et al*., 1994). The transient exposure of an F_0 generation gestating rat to vinclozolin at the time of embryonic sex determination promotes an adult-onset disease of spermatogenic defects and male subfertility in the offspring. Research has demonstrated that 90% of all male progeny for four generations (F_1–F_4) developed spermatogenic defects following the direct exposure of the F_0 gestating rat (Anway *et al*., 2005). This transgenerational phenotype was only transmitted through the male germ line (i.e. spermatozoon) and was not passed through the female germ line (i.e. oocyte). In young adult males, prior to 120 days of age, the primary disease phenotype was a spermatogenic cell defect in the male testis (Anway *et al*., 2005, 2006b). However, when the animals were allowed to age up to 14 months, additional transgenerational disease phenotypes developed at increased frequencies including 15% tumour development, 50% prostate disease, 35% kidney disease, 30% immune abnormalities and 25% spermatogenic defects in males from F_1–F_4 generations (Anway *et al*., 2006a). Female animals were also found to develop transgenerational disease including tumours and kidney disease (Anway *et al*., 2006a). Furthermore, the testis phenotype was also promoted by the transient embryonic exposure to the pesticide methoxychlor, which contains a mixture of metabolites with oestrogenic, anti-oestrogenic and antiandrogenic activities (Anway *et al*., 2005).

The ability of endocrine disruptors to promote adult-onset disease has been discussed previously (Gluckman *et al*., 2004). Endocrine disruptors are a large class of environmental toxicants ranging from plastics to pesticides (Heindel, 2005). These environmental toxicants generally do not promote DNA sequence mutations, which generally occur at a frequency lower than 0.01% (Barber *et al*., 2002). The frequency of the transgenerational phenotype described above (occurring in 30–90% of the animals) also could not be attributed to DNA sequence mutations. Therefore, the hypothesis was developed that the induced transgenerational phenotype is likely to be epigenetic in origin, resulting from changes in gene function that are not related to a specific DNA sequence mutations (Anway *et al*., 2005; Anway and Skinner, 2006). Epigenetic or non-genomic inheritance clearly occurs and has impacts on health and disease (Rakyan and Beck, 2006; Gluckman *et al*., 2007; Jass, 2007). The environment has the ability to regulate the epigenome that subsequently influences genome activity and disease susceptibility (Whitelaw and Whitelaw, 2006; Jirtle and Skinner, 2007). Although observations demonstrate these epigenetic transgenerational phenotypes exist, the impact they have on health and disease remains to be elucidated. The identification of epigenetic biomarkers correlated to disease could provide early stage diagnostic markers to allow preventative medicine strategies to be developed.

This transgenerational phenomenon demonstrates an epigenetic mechanism by which environmental toxicants may promote transgenerational phenotypes and adult-onset disease (Gluckman and Hanson, 2004; Heindel, 2005). A large number of studies have demonstrated that embryonic or post-natal exposures can induce adult-onset disease. The mechanism for this fetal basis of adult-onset disease is unknown, but is likely to involve epigenetic alterations in the genome (Dolinoy *et al*., 2007; van Vliet *et al*., 2007). Many adult-onset disease phenotypes are not transgenerational, but manifest in the exposed individuals. These individual disease exposures and phenotypes may also involve epigenetic mechanisms. A recent study demonstrated a neonatal exposure to bisphenol A altered DNA methylation of a number of genes and promoted an increased frequency of prostate disease in the adult (Ho *et al*., 2006). Therefore, an embryonic, post-natal or adult exposure could cause an epigenetic event that then alters the physiology of a tissue and promotes disease. It is likely that rapidly developing tissues will be more sensitive to environmental exposures and epigenetic modifications.

Acknowledgements

We acknowledge the assistance of Ms Jill Griffin and Ms Rochelle Pedersen in the preparation of the manuscript. This research was supported in part by grants from the USA National Institutes of Health, NIH/NIEHS to MKS.

References

Anway MD, Skinner MK 2006 Epigenetic transgenerational actions of endocrine disruptors. *Endocrinology* **147**(Suppl. 6) S43–S49.

Anway MD, Leathers C, Skinner MK 2006a Endocrine disruptor vinclozolin induced epigenetic transgenerational adult-onset disease. *Endocrinology* **147**, 5515–5523.

Anway MD, Memon MA, Uzumcu M, Skinner MK 2006b Transgenerational effect of the endocrine disruptor vinclozolin on male spermatogenesis. *Journal of Andrology* **27**, 868–879.

Anway MD, Cupp AS, Uzumcu M, Skinner MK 2005 Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* **308**, 1466–1469.

Barber R, Plumb MA, Boulton E *et al*. 2002 Elevated mutation rates in the germ line of first- and second-generation offspring of irradiated male mice. *Proceedings of the National Academy of Sciences* of the USA **99**, 6877–6882.

Beaujean N, Taylor JE, McGarry M *et al*. 2004 The effect of interspecific oocytes on demethylation of sperm DNA. *Proceedings of the National Academy of Sciences* of the USA **101**, 7636–7640.

Dolinoy DC, Weidman JR, Jirtle RL 2007 Epigenetic gene regulation: linking early developmental environment to adult disease. *Reproductive Toxicology* **23**, 297–307.

Feinberg AP 2004 The epigenetics of cancer etiology. *Seminars in Cancer Biology* **14**, 427–432.

Fulka H, Mrazek M, Tepla O, Fulka J Jr 2004 DNA methylation pattern in human zygotes and developing embryos. *Reproduction* **128**, 703–708.

Gluckman PD, Hanson MA 2004 Developmental origins of disease paradigm: a mechanistic and evolutionary perspective. *Pediatric Research* **56**, 311–317.

Gluckman PD, Hanson MA, Beedle AS 2007 Non-genomic transgenerational inheritance of disease risk. *Bioessays* **29**, 145–154.

Heindel JJ 2005 The fetal basis of adult disease: Role of environmental exposures – introduction. *Birth Defects Research. Part A, Clinical and Molecular Teratology* **73**, 131–132.

Herceg Z 2007 Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors. *Mutagenesis* **22**, 91–103.

Hisano M, Ohta H, Nishimune Y, Nozaki M 2003 Methylation of CpG dinucleotides in the open reading frame of a testicular germ cell-specific intronless gene, Tact1/Actl7b, represses its expression in somatic cells. *Nucleic Acids Research* **31**, 4797–4804.

Ho SM, Tang WY, Belmonte de Frausto Y, Prins GS 2006 Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. *Cancer Research* **66**, 5624–5632.

Hughes IA 2001 Minireview: sex differentiation. *Endocrinology* **142**, 3281–3287.

Jass JR 2007 Heredity and DNA methylation in colorectal cancer. *Gut* **56**, 154–155.

Jiang YH, Bressler J, Beaudet *et al*. 2004 Epigenetics and human disease. *Annual Review of Genomics and Human Genetics* **5**, 479–510.

Jirtle RL, Skinner MK 2007 Environmental epigenomics and disease susceptibility. *Nature Reviews Genetics* **8**, 253–262.

Kanai Y, Hiramatsu R, Matoba S, Kidokoro T 2005 From SRY to SOX9: mammalian testis differentiation. *Journal of Biochemistry (Tokyo)* **138**, 13–19.

Kelce WR, Monosson E, Gamcsik MP *et al*. 1994 Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites. *Toxicology and Applied Pharmacology* **126**, 276–285.

Li JY, Lees-Murdock DJ, Xu GL, Walsh CP 2004 Timing of establishment of paternal methylation imprints in the mouse. *Genomics* **84**, 952–960.

Muhle R, Trentacoste SV, Rapin I 2004 The genetics of autism. *Pediatrics* **113**, e472–486.

Nishino K, Hattori N, Tanaka S, Shiota K 2004 DNA methylation-mediated control of *Sry* gene expression in mouse gonadal development. *Journal of Biological Chemistry* **279**, 22306–22313.

Rakyan VK, Beck S 2006 Epigenetic variation and inheritance in mammals. *Current Opinion in Genetics and Development* **16**, 573–577.

Robertson KD 2005 DNA methylation and human disease. *Nature Reviews Genetics* **6**, 597–610.

Schulz WA, Hatina J 2006 Epigenetics of prostate cancer: beyond DNA methylation. *Journal of Cellular and Molecular Medicine* **10**, 100–125.

Steele W, Allegrucci C, Singh R *et al*. 2005 Human embryonic stem cell methyl cycle enzyme expression: modelling epigenetic programming in assisted reproduction? *Reproductive BioMedicine Online* **10**, 755–766.

Tarozzi N, Bizzaro D, Flamigni C, Borini A 2007 Clinical relevance of sperm DNA damage in assisted reproduction. *Reproductive BioMedicine Online* **14**, 746–757.

van Vliet, J, Oates NA, Whitelaw W 2007 Epigenetic mechanisms in the context of complex diseases. *Cellular and Molecular Life Sciences* **64**, 1531–1538.

Weidman JR, Dolinoy DC, Murphy SK, Jirtle RL 2007 Cancer susceptibility: epigenetic manifestation of environmental exposures. *Cancer Journal* **13**, 9–16.

Whitelaw NC, Whitelaw E 2006 How lifetimes shape epigenotype within and across generations. *Human Molecular Genetics* **15**, R131–137.

Yamazaki Y, Mann MR, Lee SS *et al*. 2003 Reprogramming of primordial germ cells begins before migration into the genital ridge, making these cells inadequate donors for reproductive cloning. *Proceedings of the National Academy of Sciences* of the USA **100**, 12207–12212.

Yang J, Yang S, Beaujean N *et al*. 2007 Epigenetic marks in cloned rhesus monkey embryos: comparison with counterparts produced *in vitro*. *Biology of Reproduction* **76**, 36–42.

Received 2 July 2007; refereed 30 July 2007; accepted 14 September 2007.