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Hsp90 and environmental impacts on epigenetic states: a model for the trans-generational effects of diethylstibesterol on uterine development and cancer

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Hsp90 is a chaperone for over 100 'client proteins' in the cell, most of which are involved in signaling pathways. For example, Hsp90 maintains several nuclear hormone receptors, such as the estrogen receptor (ER), as agonist-receptive monomers in the cytoplasm. In the presence of agonist, Hsp90 dissociates and the receptors dimerize, enter the nucleus and ultimately activate transcription of the target genes. Increasing evidence suggests that Hsp90 also has a role in modifying the chromatin conformation of many genes. For example, Hsp90 has recently been shown to increase the activity of the histone H3 lysine-4 methyltransferase SMYD3, which activates the chromatin of target genes. Further evidence for chromatin-remodeling functions is that Hsp90 acts as a capacitor for morphological evolution by masking epigenetic variation. Release of the capacitor function of Hsp90, such as by environmental stress or by drugs that inhibit the ATP-binding activity of Hsp90, exposes previously hidden morphological phenotypes in the next generation and for several generations thereafter. The chromatin-modifying phenotypes of Hsp90 have striking similarities to the trans-generational effects of the ER agonist diethylstilbesterol (DES). Prenatal and perinatal exposure to DES increases the predisposition to uterine developmental abnormalities and cancer in the daughters and granddaughters of exposed pregnant mice. In this review, we propose that trans-generational epigenetic phenomena involving Hsp90 and DES are related and that chromatin-mediated WNT signaling modifications are required. This model suggests that inhibitors of Hsp90, WNT signaling and chromatin-remodeling enzymes might function as anticancer agents by interfering with epigenetic reprogramming and canalization in cancer stem cells.

CANALIZATION IN DEVELOPMENT AND CANCER

In 1942, Conrad Waddington popularized the term 'canalization' in his classic *Nature* letter, 'Canalization of development and the inheritance of acquired characters' (1). He wrote, 'The main thesis is that developmental reactions, *as they occur in organisms submitted to natural selection*, are in general canalized. That is to say, they are adjusted so as to bring about one definite end-result regardless of minor variations in conditions during the course of the reaction' (1). A morphological phenotype, such as a novel adaptive character, is 'canalized' through a process called 'assimilation', which is defined as a selection of existing genetic variation in a population over several generations until the phenotype is stabilized or 'fixed' (1). In 1953, Waddington (2) proved that assimilation leads to canalization. Waddington stressed *Drosophila* larvae to generate a novel 'crossveinless' phenotype in which one of the crossveins in an adult wing is missing. By selecting flies in an outbred population that had the induced crossveinless phenotype, repeating the stress in their larval progeny and again selecting for crossveinless progeny, Waddington showed that the crossveinless phenotype can be 'fixed' in ~100% of the progeny

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after 10–15 generations, even in the absence of additional environmental stress (2). In other words, the novel crossveinless phenotype was 'canalized' in the 'assimilated' population.

In 1998, Rutherford and Lindquist (3) proposed a molecular mechanism explaining how stress, such as heat shock, can reveal previously hidden cryptic phenotypic variation. They showed that mutations in *Drosophila* Hsp90, or pharmacological inhibition of Hsp90 with the potent and specific inhibitor geldanamycin, reveal previously hidden phenotypic variation in diverse adult body parts such as wings, legs and eyes (3). They then performed a variation on Waddington's assimilation experiment and showed that several of these novel phenotypes can be selected for several generations until they are present, i.e., 'assimilated', even when Hsp90 activity is restored (3). Although Rutherford and Lindquist did not identify the targets of Hsp90 in their assimilation experiments in *Drosophila* (3) and later in *Arabidopsis* (4), the fact that Hsp90 has over 100 identified 'client proteins', most of which are signaling proteins (5), suggesting that Hsp90 could affect the morphological development of numerous body parts.

We expanded on assimilation studies of Rutherford and Lindquist that show that Hsp90 can function as a 'genetic capacitor' (3) by showing that Hsp90 can also function as an 'epigenetic capacitor' for phenotypic variation (6). A 'genetic capacitor', a term coined by Rutherford and Lindquist (3), is like an electric capacitor that instead of storing electrical potential energy for later release, stores morphological variation for later release. An 'epigenetic capacitor', we propose, stores epigenetic potential for morphological variation (6). For both types of capacitors, stress releases the potential for morphological variation by functionally inactivating Hsp90 (3,6).

We showed that mutations in *Drosophila* Hsp90, or pharmacological inhibition of Hsp90 with geldanamycin, induce ectopic large bristle outgrowths (ELBOs) from the eyes of 'epigenetically sensitized' isogenized flies with the $Kr^{If\text{-}1}$ mutation (Fig. 1). An 'epigenetically sensitized' fly is a term that we coined which refers to a mutation that does not yet induce a new morphological phenotype, but it is on the verge of producing a new morphological phenotype (6). Flies with the $Kr^{If\text{-}1}$ mutation will have the ELBO phenotype if the chromatin is altered epigenetically, such as by inactivation of Hsp90 or by reducing the amount of other chromatin-remodeling genes in the mother (6). This term is analogous to the term 'genetically sensitized', which is a type of animal that is 'sensitized' for new mutations in a particular signaling pathway because it is heterozygous for a mutation in a gene encoding a component in the same pathway (7).

$Kr^{If\text{-}1}$ flies, which have the zinc-finger containing Krüppel (Kr) transcription factor ectopically expressed in the eye imaginal discs, presumably by having an enhancer element inserted at the *Kr* locus (8), normally have a reduced number of eye facets but no ELBOs (6). In our paper, we showed that 'epigenetic assimilation' can occur by selecting for flies with the ELBO phenotype, even in the absence of genetic variation (6). After five or six generations of selection, 60–70% of the progeny had the ELBO phenotype, even though geldanamycin was only added in the parental generation (6). The study of Lindquist and coworkers and our studies have been the subject of several recent reviews and commentaries (9–12).

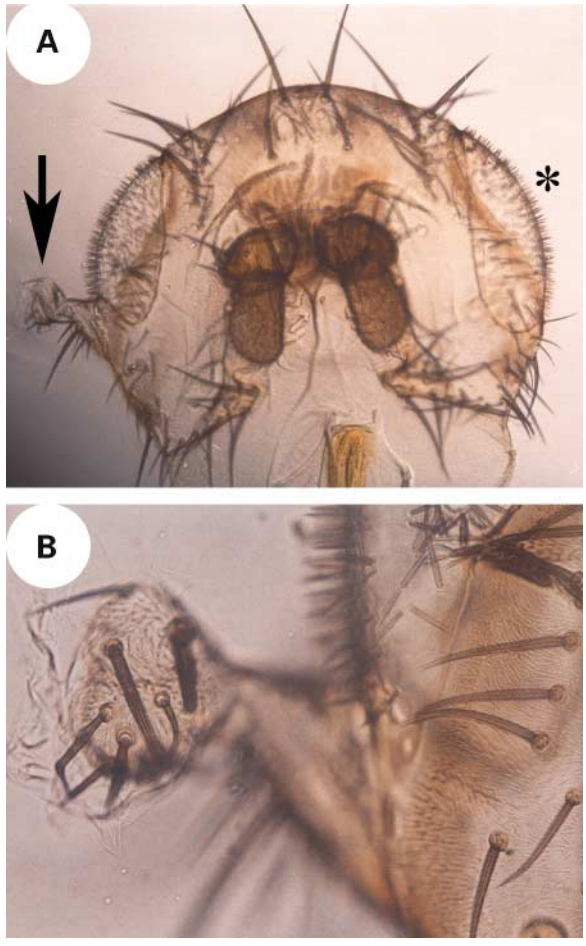

Figure 1. Fly head with a ELBO. (**A**) Arrow points to the ELBO and the asterix indicates a $Kr^{If\text{-}1}/+$ eye without an ELBO. (**B**) Enlargement of the ELBO shown in (A).

In this review, we call the stability of epigenetically assimilated traits, such as the ELBO phenotype, 'epigenetic canalization'. We propose that progression of cancer is another example of 'epigenetic canalization' because inheritance of tumor-promoting 'metastable epi-alleles', genes with a stochastic distribution of methylation states (13), are selected in precancer cells as they progress to a malignant state (14,15). Here, we argue that trans-generational effects of diethylstilbestrol (DES), a synthetic estrogen, exposure on uterine cancer and development, and cancer epigenesis in general, have many similarities to the trans-generational 'epigenetic assimilation' phenomena described earlier. Although technically 'assimilation' experiments require selection of a phenotype for several generations (2), whereas the DES effect is only observed in the children and grandchildren, we speculate that DES exposure can be the first step in an 'epigenetic assimilation' process, which might last for numerous generations. In the next section, we will review the trans-generational effects of DES exposure, and in the final section, we will attempt a 'conceptual assimilation' of these studies to form a unified

model on how DES affects uterine development and cancer in a trans-generational manner.

TRANS-GENERATIONAL EPIGENETIC EFFECTS MEDIATED BY DES

Between 1947 and 1971, over 1 million American women were exposed to DES when they took the drug to reduce the risk of mis-carriage (16). DES in meat products caused further human exposure because this drug was widely fed to beef cattle and other livestock to accelerate their growth (17). DES was present at biologically relevant levels in beef, and DES is metabolically very stable, so this was likely a significant source of exposure in humans (17). In 1971, an estimated 27 600 kg of DES was used in livestock feed lots (17).

Daughters of mothers exposed to DES during the first trimester often show developmental abnormalities in the structure of the uterus, cervix or vagina and an increased risk in developing a rare form of cancer, clear-cell adenocarcinoma (16). Studies with laboratory animals, described subsequently, have confirmed these early observations on the adverse effects of DES on reproductive system development and cancer. However, the exact risk of DES exposure in humans is still not clear. For example, in a recent study on DES exposure in humans, Robboy's laboratory, which has been studying DES exposure in humans for >30 years, concludes, 'The findings support an association between *in utero* DES exposure and high-grade squamous neoplasia, although a role for more intensive screening among DES-exposed women in the production of this excess could not be completely ruled out' (18).

Perinatal (during the first 3 months after birth) exposure to DES induces developmental abnormalities in the reproductive tract of rodents such as mouse (19–21), rat (22,23), and hamsters (24,25). For example, when neonatal mice are exposed to DES, 2 μg/pup per day for the first 5 days of life, ~90% of the mice develop uterine cancer by 18 months (20). Most strikingly, using this and similar neonatal exposure paradigms in mice, DES affects uterine development in a trans-generational manner in the daughters of the neonatally exposed female mice and the granddaughters of DES-exposed pregnant mice (26–29). Therefore, even though DES has not been used commercially in the USA since the 1970s, nevertheless these results suggest that another generation of humans is at risk from DES exposure in their grandmothers. If they have not already begun, it is now time to begin studies on the possible effects of DES on the granddaughters of women exposed in the mid-20th century.

Although, to our knowledge, the mechanisms for the trans-generational effects of DES exposure have not yet been investigated, the effects of neonatal exposure to DES on uterine cancer in the same generation have been shown to be mediated, at least partly, by altering the CpG methylation pattern of key uterine cancer genes in mice. For example, Li *et al.* (16,30) have recently shown that neonatal exposure of DES in mice, using the previously mentioned conditions, induces persistent increases in *c-fos* mRNA expression and hypomethylation of specific enhancer-binding sites. Altered DNA methylation by DES is probably a gene-specific phenomenon because the CpG methylation of the promoters of the *Hox-a10* and *Hox-a11* genes are not altered by neonatal exposure to DES, even though DES dramatically downregulates expression of these genes (31). It is possible that CpG methylation alterations in many of the key uterine cancer genes are stable during gametogenesis and that this might explain the trans-generational effects of DES exposure on uterine development.

Several studies have suggested that CpG methylation regulates modifications of the histones around the CpG methylated genes (32–34). For example, expression of mammalian Dnmt3a in *Drosophila* increases histone H3 lysine-9 methylation (35), which is catalyzed by the Su(var)3-9 protein. This suggests that histone H3 lysine-9 methylation and DNA methylation are linked in *Drosophila* and likely also in mammals (36). Consistent with this, human cells deficient in *Dnmt1* have recently been shown to have altered histone H3 modification patterns (37).

Figure 2 summarizes the role of Hsp90 in regulating the estrogen receptor (ER), with an emphasis on the chromatin remodeling that occurs at ER-target genes. In the absence of estrogen, a monomer of the ER is bound to a dimer of Hsp90 in an inactive, but agonist-receptive complex (Fig. 2A) (5,38). In the presence of estrogen or DES, Hsp90 releases ER and ligand-bound ER dimerizes and enters the nucleus where it activates transcription of target genes (Fig. 2B) (5,38). Furthermore, Freeman and Yamamoto (39) showed that Hsp90 is associated with the chromatin and regulates inactivation of the ER chromatin complex when the ligand is removed. Presumably, either Hsp90a or Hsp90b isoform in humans can have these functions (39). CARM1, a histone H3 arginine-17 methyltransferase, is one of several co-activators, which associates with the ER on target genes (Fig. 2B) (40). CARM1 also activates the chromatin of target genes by recruiting the histone acetyltransferase CBP (41,42). Recently, histone deimination by PAD4 has been shown to antagonize arginine methylation by converting the methyl-arginine to citruilline, thereby converting chromatin that supports transcription to chromatin which does not support transcription (43,44). Although, to our knowledge, there is no evidence for this yet, we speculate that PAD4 is used to inactivate the chromatin at ER target genes in the absence, or after the removal, of estrogen or DES (Fig. 2A).

In Figure 2, we also speculate on how Hsp90 might regulate expression of the WNT genes in an opposite manner as the ER-target genes. The WNT family instructs a wide array of cell behavioral changes and morphogenetic events that contribute to specify the position and shape of a variety of organs, tissues and structures during normal development and in tumors (reviewed in 45). Several WNTs, such as Wnt7a, are required for postnatal and estrogen-mediated growth of the female reproductive tract by suppressing uterine cell death in the absence of estrogen (46). Conversely to ER, where Hsp90 maintains the ER in an agonist-competent inactive state, Hsp90 is required for optimal activity of the histone H3 lysine-4 methyltransferase SMYD3 (47,48). SMYD3 is required for activation of Wnt10b and possibly other WNTs (Fig. 2A) (48). We speculate that stress, and possibly also estrogen or DES, might disrupt association of SMYD3 with Hsp90 by functionally titrating Hsp90 activity

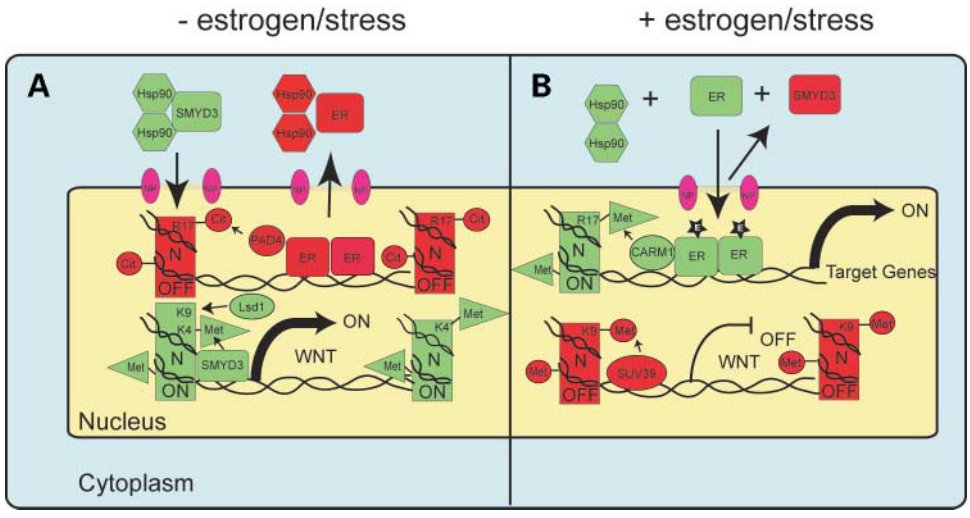

Figure 2. Effects of estrogen and stress on activation of the ER and repression of the WNT genes. (**A**) In the absence of estrogen or stress, a dimer of Hsp90 binds to a monomer of the ER and, we propose, a monomer of the histone H3 lysine methyltransferase SMYD3. SMYD3 is activated by Hsp90, and activated SMYD3 enters the nucleus and activates transcription of target genes such as WNT genes. Hsp90 forms a complex with ER and the methyl-arginine deimination enzyme PAD4, which inactivates the chromatin of the ER-target genes. (**B**) In the presence of estrogen or stress, the ER dimerizes, enters the nucleus and binds to chromatin activating enzymes such as the histone H3 arginine-17 methyltransferase CARM1. In the presence of estrogen, the ER-target genes are activated. We propose that the WNT genes are not expressed because SMYD3 cannot be activated when estrogen or stress is present. Chromatin inactivating enzymes, such as the histone H3 lysine-9 methyltransferase Suv39, could inactivate the chromatin of the WNT genes. Lsd1 can remove the methyl group on methyl-lysine (52) and can therefore presumably counteract the effect of Suv39. Active proteins and histones in chromatin that can support transcription are indicated (green). Likewise, inactive proteins and histone in regions that cannot support transcription are also indicated (red).

(3), therefore, inactivating SMYD3 so that it can no longer mediate the activation of transcription of WNT genes (Fig. 2, right).

Alternatively, or additionally, to the role of SMYD3 in regulating WNT gene expression in the uterus, agonist-bound ER might form an inhibitory chromatin complex with the WNT-target genes. A precedent for this model is that the vitamin D nuclear receptor can form both activating chromatin complexes with the HAT CBP and inhibitory chromatin complexes with the histone inhibitory factors, HDAC1 and HDAC2, among others, depending on whether vitamin D is present (49,50). Similarly, Klinge *et al.* (51) have recently shown that the sequence of the estrogen response element (ERE) determines whether ER forms an activating chromatin complex with CARM1 (among others) or an inhibitory chromatin complex with nuclear receptor co-repressor and silencing mediator for retinoid and thyroid hormone receptor (SMRT). We speculate that WNT genes which regulate uterine development might also have inhibitory EREs, but this has not yet been tested.

Although many of the aspects of our model in Figure 2 are highly speculative, a key point of the model is the reversibility of activating and inactivating chromatin by enzymes that counteract each other's functions, possibly in the same complex. In this paragraph, we will specify which aspects of Figure 2 are supported by evidence and which aspects are more speculative. For example, there is evidence that CARM1 is an ER co-activator that methylates histone H3 arginine-17 (40), but there is no evidence yet to our knowledge that PAD4, which converts this methyl-arginine to citrulline, is present on this complex. In addition, there is no evidence to our knowledge that Su(var)3-9, a histone H3 lysine-9 methyltransferase, is involved in repressing Wnt transcription when estrogen is present, nor is there evidence that Lsd1, which is a lysine demethylase (52), re-activates Wnt transcription when estrogen is absent.

One stimulus for making this 'yin-and-yang' or 'Voldemort-type' model (53), which has opposing activities on the same complex, is the recent finding from Kudlow's laboratory that *O*-GlcNAc transferase (OGT) and NCOAT (nuclear cytoplasmic *O*-GlcNAcase and acetyltransferase) are on the same chromatin-remodeling complex (54). Modification of chromatin proteins, such as the transcription factor SP1, by *O*-GlcNAc occurs in high-glucose conditions, whereas NCOAT is active under low-glucose conditions (54). *O*-GlcNAc modifications have been proposed to be a 'nutrient sensor', which regulates the activity of numerous transcription factors, in addition to SP1 (55).

TRANS-GENERATIONAL EPIGENETICS: A UNIFYING MODEL

As noted earlier, the trans-generational effects that we observe with the ELBO phenotype (Fig. 1) and DES-induced uterine abnormalities have several similarities. First, both phenomena involve in the signaling chaperone Hsp90. Geldanamycin, a specific Hsp90 inhibitor induces the ELBO phenotype in the $Kr^{If\text{-}1}$ genetic background (6) and Hsp90 is required to maintain the ER in a agonist-receptive inactive cytoplasmic form (Fig. 2) (5,38). Secondly, chromatin remodeling is evidently involved in both processes. The histone deactylase inhibitors trichostatin A and sodium butyrate partially suppress the ELBO phenotype (6) and nuclear receptors form complexes

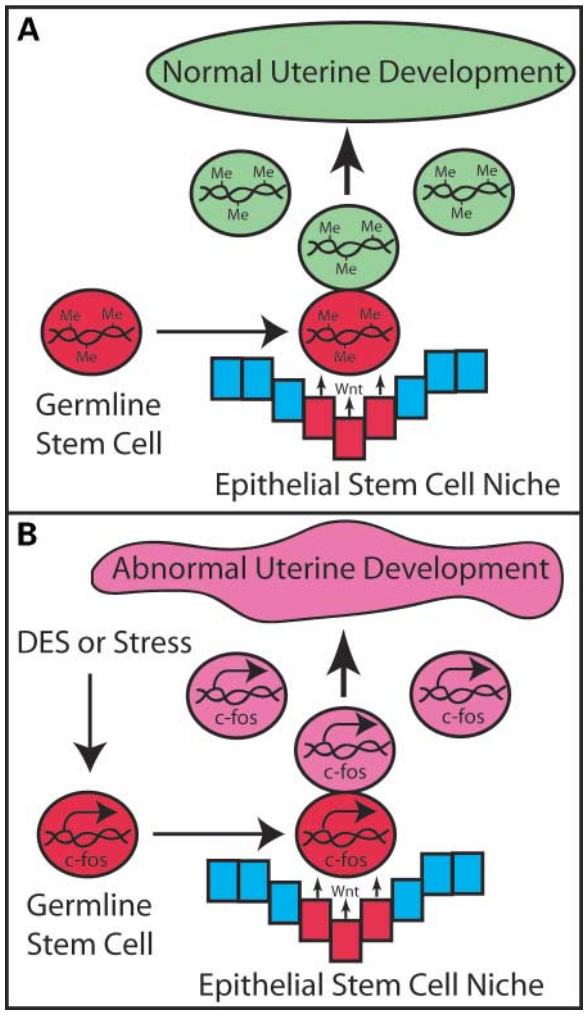

Figure 3. Model for trans-generational inheritance of DES-induced susceptibility to uterine cancer. Methylated CpG sites in the DNA are indicated (Me).

with histone deacetylases (HDACs) and histone acetyltransferases on the chromatin (49,50). We also have evidence that mutations in several histone-modifying enzymes, such as the histone methyltransferase Su(var)3-9 and the HDAC Rpd3, suppress the ELBO phenotype when there is a deficiency in the levels of these proteins in the mother (D.M. Ruden and coworkers, unpublished data). Finally, Wnt signaling is involved in both processes. Mutations in WNT-pathway genes suppress the ELBO phenotype (D.M. Ruden and coworkers, unpublished data), and as discussed earlier, postnatal uterine development and growth requires WNT signaling (46). For example, Wnt7a and other members of this family are required for guiding the epithelial–mesenchymal interactions, which direct postnatal uterine development (46). Lindquist and coworkers (11) also speculated on the role of WNT signaling in generating the ELBO trans-generational epigenetic phenotype.

What is the role of WNT signaling in trans-generational epigenetic phenomena such as the ELBO phenotype (Fig. 1) and those mediated by DES? The hypothesis of this review is that WNT is required for epigenetic reprograming of stem cells in both phenomena (Fig. 3). Germline stem cells must be involved in the epigenetic transmission of the ELBO phenotype because the phenotype is transmitted through both the male and female germlines (6). Epithelial stem cells are also probably involved in generating the ELBO phenotype because the bristle outgrowths are likely derived from trans-differentiated epithelial stem cells in the eye imaginal discs (6). As are all stem cells, epithelial stem cells derived from germline stem cells and epithelial stem cells such as those in the epidermis, gut and uterus, require WNT signaling for self-renewal (56,57).

In our model for the trans-generational effects of DES, we propose that under normal situations, specific chromatin regions in germline stem cells are methylated at CpG sites, and therefore, inactivated at critical enhancer regions for genes that promote uterine cancer, such as *c-fos* (Fig. 3A) (16,30). DES exposure or stress induces hypomethylation of the uterine cancer promoting genes, thereby allowing the enhancer proteins to bind to the regulatory elements and activate transcription of these genes (Fig. 3B). The germline stem cells, which are totipotent because they can become all of the cells in the organism, differentiate into pleuripotent cells such as the epithelial stem cells in the uterus (58). Although this has not yet been demonstrated, we speculate that the CpG methylation pattern in epithelial stem cells is maintained by Wnt signaling in the stem cell niches in the uterus (Fig. 3A).

As mentioned earlier, WNT signaling is required for renewal of the uterine stem cells. We propose that WNT signaling might also be required for maintenance of the CpG methylation status of the uterine cancer promoting genes, and thereby contribute to the trans-generational effects of DES and stress. Further experiments are required to test the various aspects of this model. This model suggests that inhibitors of Hsp90, WNT signaling and chromatin-remodeling enzymes might function as anticancer agents by interfering with epigenetic reprograming and canalization in cancer stem cells. We hope that this review stimulates further research in this, in our view, understudied area.

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REFERENCES

1. Waddington, C.H. (1942) Canalization of development and the inheritance of acquired characters. *Nature*, **150**, 563–565.
2. Waddington, C.H. (1953) Genetic assimilation of an acquired character. *Evolution*, **7**, 118–126.
3. Rutherford, S.L. and Lindquist, S. (1998) Hsp90 as a capacitor for morphological evolution. *Nature*, **396**, 336–342.
4. Queitsch, C., Sangster, T.A. and Lindquist, S. (2002) Hsp90 as a capacitor of phenotypic variation. *Nature*, **417**, 618–624.
5. Pratt, W.B. and Toft, D.O. (2003) Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp. Biol. Med.*, **228**, 111–133.
6. Sollars, V., Lu, X., Xiao, L., Wang, X., Garfinkel, M.D. and Ruden, D.M. (2003) Evidence for an epigenetic mechanism by which Hsp90 acts as a capacitor for morphological evolution. *Nat. Genet.*, **33**, 70–74.

7. Glasscock, E. and Tanouye, M.A. (2005) *Drosophila* couch potato mutants exhibit complex neurological abnormalities including epilepsy phenotypes. *Genetics*, in press.
8. Carrera, P., Abrell, S., Kerber, B., Walldorf, U., Preiss, A., Hoch, M. and Jackle, H. (1998) A modifier screen in the eye reveals control genes for Kruppel activity in the *Drosophila* embryo. *Proc. Natl. Acad. Sci. USA*, **95**, 10779–10784.
9. Ruden, D.M., Garfinkel, M.D., Sollars, V.E. and Lu, X. (2003) Waddington's widget: Hsp90 and the inheritance of acquired characters. *Semin. Cell Dev. Biol.*, **14**, 301–310.
10. Rutherford, S.L. and Henikoff, S. (2003) Quantitative epigenetics. *Nat. Genet.*, **33**, 6–8.
11. Sangster, T.A., Queitsch, C. and Lindquist, S. (2003) Hsp90 and chromatin: Where is the link? *Cell Cycle*, **2**, 166–168.
12. Pigliucci, M. (2003) Epigenetics is back! Hsp90 and phenotypic variation. *Cell Cycle*, **2**, 34–35.
13. Rakyan, V.K., Blewitt, M.E., Druker, R., Preis, J.I. and Whitelaw, E. (2002) Metastable epialleles in mammals. *Trends Genet.*, **18**, 348–351.
14. Bjornsson, H.T., Fallin, M.D. and Feinberg, A.P. (2004) An integrated epigenetic and genetic approach to common human disease. *Trends Genet.*, **20**, 350–358.
15. Feinberg, A.P. and Tycko, B. (2004) The history of cancer epigenetics. *Nature Rev. Cancer*, **4**, 143–153.
16. Li, S., Hursting, S.D., Davis, B.J., McLachlan, J.A. and Barrett, J.C. (2003) Environmental exposure, DNA methylation, and gene regulation: Lessons from diethylstilbesterol-induced cancers. *Ann. N.Y. Acad. Sci.*, **983**, 161–169.
17. Knight, W.A., III, Osborne, C.K., Yochmowitz, M.G. and McGuire, W.L. (1980) Steroid hormone receptors in the management of human breast cancer. *Ann. Clin. Res.*, **12**, 202–207.
18. Hatch, E.E., Herbst, A.L., Hoover, R.N., Noller, K.L., Adam, E., Kaufman, R.H., Palmer, J.R., Titus-Ernstoff, L., Hyer, M., Hartge, P. *et al.* (2001) Incidence of squamous neoplasia of the cervix and vagina in women exposed prenatally to diethylstilbestrol (United States). *Cancer Causes Control*, **12**, 837–845.
19. McLachlan, J.A., Newbold, R.R. and Bullock, B.C. (1980) Long-term effects on the female mouse genital tract associated with prenatal exposure to diethylstilbestrol. *Cancer Res.*, **40**, 3988–3999.
20. Newbold, R.R., Bullock, B.C. and McLachlan, J.A. (1990) Uterine adenocarcinoma in mice following developmental treatment with estrogens: a model for hormonal carcinogenesis. *Cancer Res.*, **50**, 7677–7681.
21. Iguchi, T. and Takasugi, N. (1987) Postnatal development of uterine abnormalities in mice exposed to DES *in utero. Biol. Neonate*, **52**, 97–103.
22. Branham, W.S., Zehr, D.R., Chen, J.J. and Sheehan, D.M. (1988) Uterine abnormalities in rats exposed neonatally to diethylstilbestrol, ethynylestradiol, or clomiphene citrate. *Toxicology*, **51**, 201–212.
23. Medlock, K.L., Branham, W.S. and Sheehan, D.M. (1992) Long-term effects of postnatal exposure to diethylstilbestrol on uterine estrogen receptor and growth. *J. Steroid Biochem. Mol. Biol.*, **42**, 23–28.
24. Leavitt, W.W., Evans, R.W. and Hendry, W.J., III. (1981) Etiology of DES-induced uterine tumors in the Syrian hamster. *Adv. Exp. Med. Biol.*, **138**, 63–86.
25. Gilloteaux, J. and Steggles, A.W. (1985) Endometrium cell surface abnormalities in the Syrian hamster as a result of *in utero* exposure to diethylstilbestrol. *Scan. Electron Microsc.*, **1**, 303–309.
26. Newbold, R.R., Hanson, R.B., Jefferson, W.N., Bullock, B.C., Haseman, J. and McLachlan, J.A. (1998) Increased tumors but uncompromised fertility in the female descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis*, **19**, 1655–1663.
27. Newbold, R.R., Hanson, R.B., Jefferson, W.N., Bullock, B.C., Haseman, J. and McLachlan, J.A. (2000) Proliferative lesions and reproductive tract tumors in male descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis*, **21**, 1355–1363.
28. Turusov, V.S., Trukhanova, L.S., Parfenov Yu, D. and Tomatis, L. (1992) Occurrence of tumours in the descendants of CBA male mice prenatally treated with diethylstilbestrol. *Intl. J. Cancer*, **50**, 131–135.
29. Walker, B.E. and Haven, M.I. (1997) Intensity of multigenerational carcinogenesis from diethylstilbestrol in mice. *Carcinogenesis*, **18**, 791–793.
30. Li, S., Hansman, R., Newbold, R., Davis, B., McLachlan, J.A. and Barrett, J.C. (2003) Neonatal diethylstilbestrol exposure induces persistent elevation of c-fos expression and hypomethylation in its exon-4 in mouse uterus. *Mol. Carcinog.*, **38**, 78–84.
31. Li, S., Ma, L., Chiang, T., Burow, M., Newbold, R.R., Negishi, M., Barrett, J.C. and McLachlan, J.A. (2001) Promoter CpG methylation of Hox-a10 and Hox-a11 in mouse uterus not altered upon neonatal diethylstilbestrol exposure. *Mol. Carcinog.*, **32**, 213–219.
32. Csankovszki, G., Nagy, A. and Jaenisch, R. (2001) Synergism of Xist RNA, DNA methylation, and histone hypoacetylation in maintaining X chromosome inactivation. *J. Cell Biol.*, **153**, 773–784.
33. Biniszkiewicz, D., Gribnau, J., Ramsahoye, B., Gaudet, F., Eggan, K., Humpherys, D., Mastrangelo, M.A., Jun, Z., Walter, J. and Jaenisch, R. (2002) Dnmt1 overexpression causes genomic hypermethylation, loss of imprinting, and embryonic lethality. *Mol. Cell. Biol.*, **22**, 2124–2135.
34. Jaenisch, R. and Bird, A. (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat. Genet.*, **33** (suppl.) 245–254.
35. Weissmann, F., Muyrers-Chen, I., Musch, T., Stach, D., Wiessler, M., Paro, R. and Lyko, F. (2003) DNA hypermethylation in *Drosophila melanogaster* causes irregular chromosome condensation and dysregulation of epigenetic histone modifications. *Mol. Cell. Biol.*, **23**, 2577–2586.
36. Schneider, R., Bannister, A.J., Myers, F.A., Thorne, A.W., Crane-Robinson, C. and Kouzarides, T. (2004) Histone H3 lysine 4 methylation patterns in higher eukaryotic genes. [Erratum (2004) *Nat. Cell Biol.* **6**,465.] *Nat. Cell Biol.*, **6**, 73–77.
37. Espada, J., Ballestar, E., Fraga, M.F., Villar-Garea, A., Juarranz, A., Stockert, J.C., Robertson, K.D., Fuks, F. and Esteller, M. (2004) Human DNA methyltransferase 1 is required for maintenance of the histone H3 modification pattern. *J. Biol. Chem.*, **279**, 37175–37184.
38. Fliss, A.E., Benzeno, S., Rao, J. and Caplan, A.J. (2000) Control of estrogen receptor ligand binding by Hsp90. *J. Steroid Biochem. Mol. Biol.*, **72**, 223–230.
39. Freeman, B.C. and Yamamoto, K.R. (2002) Disassembly of transcriptional regulatory complexes by molecular chaperones. *Science*, **296**, 2232–2235.
40. Hong, H., Kao, C., Jeng, M.H., Eble, J.N., Koch, M.O., Gardner, T.A., Zhang, S., Li, L., Pan, C.X., Hu, Z. *et al.* (2004) Aberrant expression of CARM1, a transcriptional coactivator of androgen receptor, in the development of prostate carcinoma and androgen-independent status. *Cancer*, **101**, 83–89.
41. Chevillard-Briet, M., Trouche, D. and Vandel, L. (2002) Control of CBP co-activating activity by arginine methylation. *EMBO J.*, **21**, 5457–5466.
42. Daujat, S., Bauer, U.M., Shah, V., Turner, B., Berger, S. and Kouzarides, T. (2002) Crosstalk between CARM1 methylation and CBP acetylation on histone H3. (See comment). *Curr. Biol.*, **12**, 2090–2097.
43. Cuthbert, G.L., Daujat, S., Snowden, A.W., Erdjument-Bromage, H., Hagiwara, T., Yamada, M., Schneider, R., Gregory, P.D., Tempst, P., Bannister, A.J. *et al.* (2004) Histone deimination antagonizes arginine methylation. *Cell*, **118**, 545–553.
44. Wang, Y., Wysocka, J., Sayegh, J., Lee, Y.H., Perlin, J.R., Leonelli, L., Sonbuchner, L.S., McDonald, C.H., Cook, R.G., Dou, Y. *et al.* (2004) Human PAD4 regulates histone arginine methylation levels via demethylimination. *Science*, **306**, 279–283.
45. Peifer, M. and Polakis, P. (2000) Wnt signaling in oncogenesis and embryogenesis—a look outside the nucleus. *Science*, **287**, 1606–1609.
46. Carta, L. and Sassoon, D. (2004) Wnt7a is a suppressor of cell death in the female reproductive tract and is required for postnatal and estrogen-mediated growth. *Biol. Reprod.*, **71**, 444–454.
47. Sims, R.J., III and Reinberg, D. (2004) From chromatin to cancer: a new histone lysine methyltransferase enters the mix. (Comment). *Nat. Cell Biol.*, **6**, 685–687.
48. Hamamoto, R., Furukawa, Y., Morita, M., Iimura, Y., Silva, F.P., Li, M., Yagyu, R. and Nakamura, Y. (2004) SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cells. (See comment). *Nat. Cell Biol.*, **6**, 731–740.
49. Kato, S., Fujiki, R. and Kitagawa, H. (2004) Vitamin D receptor (VDR) promoter targeting through a novel chromatin remodeling complex. *J. Steroid Biochem. Mol. Biol.*, **89–90**, 173–178.
50. Huang, C., Sloan, E.A. and Boerkoel, C.F. (2003) Chromatin remodeling and human disease. *Curr. Opin. Genet. Dev.*, **13**, 246–252.

51. Klinge, C., Jernigan, S., Mattingly, K., Risinger, K. and Zhang, J. (2004) Estrogen response element-dependent regulation of transcriptional activation of estrogen receptors alpha and beta by coactivators and corepressors. *J. Mol. Endocrinol.*, **33**, 387–410.
52. Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J.R., Cole, P.A., Casero, R.A. and Shi, Y. (2004) Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell*, **119**, 941–953.
53. Rowling, J.K. and GrandPrâe, M. (1999) *Harry Potter and the Chamber of Secrets.* Arthur A. Levine Books, New York.
54. Toleman, C., Paterson, A.J., Whisenhunt, T.R. and Kudlow, J.E. (2005) Characterization of the histone acetyltransferase (HAT) domain of a bifunctional protein with activable *O*-GlcNAcase and HAT activities. *J. Biol. Chem.*, **279**, 53665–55673.
55. Zachara, N.E. and Hart, G.W. (2004) *O*-GlcNAc a sensor of cellular state: the role of nucleocytoplasmic glycosylation in modulating cellular function in response to nutrition and stress. *Biochim. Biophys. Acta*, **1673**, 13–28.
56. Reya, T., Duncan, A.W., Ailles, L., Domen, J., Scherer, D.C., Willert, K., Hintz, L., Nusse, R. and Weissman, I.L. (2003) A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature*, **423**, 409–414.
57. Reya, T., Morrison, S.J., Clarke, M.F. and Weissman, I.L. (2001) Stem cells, cancer, and cancer stem cells. *Nature*, **414**, 105–111.
58. Spradling, A., Drummond-Barbosa, D. and Kai, T. (2001) Stem cells find their niche. *Nature*, **414**, 98–104.