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Developmental Environmental Exposure Alters the Epigenetic Features of Myometrial Stem Cells

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THE BIOLOGY OF UTERINE FIBROIDS

Uterine fibroids (UFs), are the most common pelvic tumors, occurring in 70–80% of all reproductive-aged women and are the leading indication for hysterectomy worldwide.[1–3] Although UFs are benign tumors, they typically cause severe menstrual bleeding, pelvic pain, preterm labor, recurrent abortion, and infertility. Hysterectomy is currently the main treatment used in women who no longer desire childbearing.[4–6] UFs are hormonally responsive to estradiol and progesterone as well as other steroid hormones, and regress after menopause.[7] Although, the cause of UFs is largely unknown, several risk factors are linked to UF development, which include age, race and ethnicity, family history, body mass index (BMI), etc.[7,8]

THE DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

The Developmental Origins of Health and Disease (DOHaD) paradigm is one of the most rapidly growing areas of biomedical research now-a-days.[9] This research field originated with early findings that prenatal nutrition was linked with late-onset coronary heart disease[10] and malnutrition and low-level exposures to drugs and toxic substances are well tolerated by a pregnant woman, but her gestating fetus would be afflicted by adverse effects, some of which might become obvious only later in life.[11,12] The field has now broadened to encompass a variety of environmental and occupational hazards. When these environmental insults disrupt early developmental processes, they may cause permanent changes in cellular characteristics that persist and then lead to increased susceptibility to a variety of diseases later in life.

Unlike in the adult, the perinatal/neonatal organ's response to environmental exposure is much more rapid and severe.[13,14] Environmental exposure is capable of causing organism toxicity due to immature immune system, lack of deoxyribonucleic acid (DNA) repair, poor liver metabolism, and incompletely formed organ barriers in early life stage. In addition to

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CONFLICTS OF INTEREST

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toxicity, environmental exposures during critical periods of organ development can permanently reprogram normal physiological responses to increase susceptibility to diseases later in life.[15] The epigenomic programming that occurs during development exhibits a high degree of plasticity, and is modifiable by extrinsic factors such as environmental exposures.[16,17]

MOLECULAR MECHANISM OF REPROGRAMMING: ENVIRONMENTAL FACTORS ALTERS THE EPIGENOME OF UTERUS

Due to the plasticity during development of tissues and organs including uterine, accumulating evidence has shown that environmental factors act on epigenome *via* DNA methylation and histone modification, which eventually lead to alteration of gene expression pattern and related diseases later in life. In utero the exposure of bisphenol A (BPA), an organic synthetic compound belonging to the group of diphenylmethane derivatives and bisphenols, altered the global CpG methylation profile of the uterine genome and subsequent gene expression pattern. Changes in estrogen response were accompanied by altered methylation that preferentially affected estrogen receptor-α (ERα)-binding genes.[18] Neonatal exposure of CD-1 mice to diethylstilbestrol (DES), a synthetic non-steroidal estrogen of the stilbestrol group, induced uterine adenocarcinoma in aging animals, concomitantly decreasing DNA methylation of nucleosome binding protein 1 (Nsbp1) promoter CpG Island (CGI) in the uteri which leads to persistent overexpression of Nsbp1 throughout life.[16,19] Moreover, 17β-estradiol and other environmental estrogens (DES and genistein) are capable of inducing phosphoinositide 3-kinase (PI3K)/AKT non-genomic estrogen receptor signaling to the histone EZH2,[20] and therefore reduced levels of trimethylation of lysine 27 on histone H3 in hormone-responsive cells.[20,21] These studies provide a direct link between xenoestrogen-induced nuclear hormone receptor signaling and modulating of epigenetic machinery in response to environmental estrogen in UFs.

EPIGENETIC REPROGRAMMING OF STEM CELLS IN UTERINE FIBROIDS IN RESPONSE TO EARLY-LIFE EXPOSURES TO ENDOCRINE DISRUPTING CHEMICALS

For environmental diseases, a central subject to resolve is the role of stem cells in the tumorigenesis or pre-cursors of degenerative diseases. Developmental adverse exposures may affect the highly regulated differentiation of hematopoietic stem cells, and even slight changes in the feature of these cells may serve as indications of health effects that may not be observed until later in life and may be magnified during the entire life.[22] The environmental exposure directs the behavior of stem and progenitor cells, the fundamental source from which all tissues derive. However, environmental health studies are lacking on stem cells.[17] UF growth and progression depend on a specialized subpopulation of tumor cells, termed tumor initiating cells (TICs).[3,23] Thus, TICs represent a critical therapeutic target, but the molecular mechanisms that regulate them are poorly understood.

To determine the mechanism underlying increased risk of UF development at stem cell levels, we have recently determined the effect of early-life exposure to endocrine disrupting

chemicals (EDCs) on stem cell behavior as well as characterized myometrial stem cells (MSCs) as a target for ethnic and environmental factors that increase UF risk. We utilized Eker rats carrying a germ-line mutation in the tuberous sclerosis complex 2 (Tsc2) tumor suppressor gene, that are susceptible for development of UFs which share similar anatomic, histologic, and biologic features to human UFs.[24] Using this model, we isolated and characterized Stro1^{+}/CD44^{+} MSC/progenitor-like cells that give rise to UFs, which resided in the rat cervix, a hypoxic niche in the uterus.[25] These Stro-1^{+}/CD44^{+} MSCs responded to environmental cues, and expanded in response to developmental environmental exposures that promote UF development.[25]

Human female reproductive tract has been shown to be a target for developmental programming as a result of inappropriate early life hormone exposure.[17,26] Early life exposure to EDC compounds have been connected to increased risk of adult onset of UFs in women.[27,28] Minority communities are particularly at risk for hazardous environmental exposures.[29–31] However, similar inquiries in humans are lacking due to difficulties in collecting suitable human myometrial samples and/or to ascertain environmental exposures. We proposed that myometrium from a non-fibroid uterus that does not exhibit any detectable myometrial pathology (removed for benign gynecological indications such as pelvic organ prolapsed near the end of reproductive life≥45 years) would model normal myometrium (MyoN). In turn, myometrium collected from fibroid uteri, would model for at risk myometrium or myometrium that might be exposed in early life to injurious environmental hormonal disruptors or other toxins (MyoF). We collect these MyoF tissues at least 2 cm away from the closest fibroid to avoid any potential mechanical or paracrine effect of the fibroid tumor itself. We demonstrated by immunohistochemistry analysis that MyoF samples from African American and Caucasian, consistently exhibited significantly higher numbers of Stro1^{+}/CD44^{+} MSCs as compared to age-, BMI-, and menstrual cycle phase-matched MyoN tissues.[25]

Although the role of MSCs in development of UFs is extremely important,[23,32,33] the molecular mechanism underlying developmental exposure to EDCs and other toxins at MSCs levels has not been characterized before.[15] By ribonucleic acid (RNA)- sequencing analysis, we recently identified some key genes including estrogen responsive genes (ERGs) that are differentially regulated in MSCs early-life exposed to diethylstilbestrol (DES) verse control (VEH).[34] Subsequently, we performed gene set enrichment analysis on the ChIP-sequencing data and found enrichment of histone H3 trimethylated at lysine 4 (H3K4me3) (an active mark for gene transcription) at the promoters of ERGs in DES-MSCs as compared to VEH-MSCs. Furthermore, the increased expression of ERGs in DES-MSCs was positively correlated with the elevated H3K4me3 epigenetic mark.[34] Our current study suggest that early life exposure to DES during sensitive periods of uterine development increases the risk of UF development by reprogramming the epigenome of MSCs towards a pro-fibroid epigenomic landscape. Further understandings of EDC-induced epigenetic alteration and DNA mutations in MSCs have the potential to substantially advance UF research.

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