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Computational modeling and simulation of genital tubercle development

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ABSTRACT

Hypospadias is a developmental defect of urethral tube closure that has a complex etiology involving genetic and environmental factors, including anti-androgenic and estrogenic disrupting chemicals; however, little is known about the morphoregulatory consequences of androgen/estrogen balance during genital tubercle (GT) development. Computer models that predictively model sexual dimorphism of the GT may provide a useful resource to translate chemical-target bipartite networks and their developmental consequences across the human-relevant chemical universe. Here, we describe a multicellular agent-based model of genital tubercle (GT) development that simulates urethrogenesis from the sexually-indifferent urethral plate stage to urethral tube closure. The prototype model, constructed in CompuCell3D, recapitulates key aspects of GT morphogenesis controlled by SHH, FGF10, and androgen pathways through modulation of stochastic cell behaviors, including differential adhesion, motility, proliferation, and apoptosis. Proper urethral tube closure in the model was shown to depend quantitatively on SHH- and FGF10-induced effects on mesenchymal proliferation and epithelial apoptosis—both ultimately linked to androgen signaling. In the absence of androgen, GT development was feminized and with partial androgen deficiency, the model resolved with incomplete urethral tube closure, thereby providing an *in silico* platform for probabilistic prediction of hypospadias risk across combinations of minor perturbations to the GT system at various stages of embryonic development.

1. Introduction

Alterations in male urological development may be invoked by genetic errors and/or chemical disruption at critical times during embryo-fetal development, leading to clinical conditions such as microphallus (micropenis), chordee, and hypospadias [1–3]. Hypospadias is a neotenous defect in urethral tube closure during male genital tubercle (GT) development, resulting in one of the most common human birth defects (1 case per 200–300 liveborn males) [4,5]. The developmental origins of hypospadias encompass embryonic stages during which the sexually indifferent GT primordium is specified and then patterned into male or female phenotypes [6,7]. Male GT development, as for most embryology, is composed of many interacting parts (molecules, cells, tissues) in an intricate arrangement. As such, GT specification, patterning, and differentiation are precisely orchestrated by genetic pathways and cellular processes. Networks of individual interactions ultimately govern how the system behaves in response to chemical-induced perturbation. Multiscale modeling and simulation are thus an important approach for discovery and synthesis of biological

Abbreviations: AR, androgen receptor; ARE, androgen response element; BMP, bone morphogenetic protein; CM, core mesenchyme; EC, ectoderm; ECM, extracellular matrix; eUPE, excluded urethral plate endoderm; FGF10, fibroblast growth factor 10; GT, genital tubercle; HTS, high-throughput screening; LM, lumen; MCS, Monte Carlo steps; PM, preputial mesenchyme; RAR, retinoic acid receptor; RXR, retinoid X receptor; SHH, sonic Hedgehog; UPE, urethral plate endoderm; UTDs, urethral Tube Closure Defects; WNT, wingless-int.

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design principles underlying the response of complex adaptive systems to perturbation. Such is the case for developmental toxicity.

Our capacity to predict adverse developmental outcomes in a complex adaptive system utilizing computational (*in silico*) models may be advanced by knowledge-driven architectures of cellular networks that are both dynamic in their control and resilient in their response to chemical-induced perturbation. Prior to sexual differentiation, GT development is directed by a number of signaling pathways, including SHH/IHH, FGF, BMP, HOX, WNT, RAR/RXR, and ephrin/EphB2 [3,8–15]. Subsequently, the GT develops into a male or female phenotype depending on androgen production from the fetal testis [16–19] and estrogen production from maternal and other sources [20–23]. In human populations, an increased risk of hypospadias has been associated with single nucleotide polymorphisms (SNPs) in the SHH and FGF pathways [24,25]. In mouse genetic models, hypospadias has been linked to functional inactivation of morphoregulatory pathways such as SHH-FGF10 signaling [10,16] and to disruption of androgen synthesis/signaling. The latter follows from prenatal exposure to environmental chemicals that may alter endocrine balance in the developing fetus, such as bisphenol A, flutamide, phthalates, and vinclozolin [26–28]. Although disruption of androgen-responsive pathways is a primary cause of hypospadias [29–31], knocking out morphogenetic signaling pathways such as SHH/IHH or FGF10 in mice causes profound changes in GT development, including changes in epithelial structure [32], suppression of mesenchymal proliferation, and the distribution of programmed cell death (apoptosis) [3,10,16,18]. Still, relatively little is known about the morphoregulatory consequences of androgen/estrogen balance during GT development [3,32–35].

Earlier investigations have highlighted some of the interplay between androgen/estrogen balance, morphoregulatory signals, and cellular behaviors underlying the patterning and sexual dimorphism of GT development. For example, normal urethral development in male embryos requires closure and separation of the urethral plate endoderm from the overlying ectoderm on the ventral surface of the GT. This process is linked to sexually-dimorphic patterns of programmed cell death (apoptosis) and the local regulation of mesenchymal cell proliferation by signaling pathways such as hedgehog (SHH/IHH), FGF (FGF8, FGF10), WNT and BMP [3,35–38]. Other investigations have focused on the integration of functional genetics and epidemiology [1,2,29,33,39–42]. These are primarily qualitative models that detail the mechanisms of GT development based on functional tests of specific molecular pathways, but they lack predictive power. None provides a quantitative platform for evaluating hypotheses and generating experimentally testable predictions.

Previously, we used a systems toxicology approach [43] to identify significant correlations between environmental chemicals, molecular targets, and adverse outcomes across a broad chemical landscape with emphasis on developmental toxicity of the male reproductive system in the ToxCast library [44]. That study demonstrated a phenotypic hierarchy of testicular atrophy, sperm effects, tumors, and malformations that, in composite resembled the human Testicular Dysgenesis Syndrome (TDS) [45]. A subset of 54 chemical compounds with male developmental consequences had *in vitro* bioactivity on molecular targets that could be condensed into 156 gene annotations in a 'chemical-target bipartite network'. Although hypospadias was only one endpoint in the study, the model supported the known role of androgen and estrogen signaling pathways in the TDS hypothesis and expanded the list of molecular targets to include vascular remodeling proteins, G-protein coupled receptors (GPCRs), cytochrome-P450s, and retinoic acid signaling [44].

Given the biological complexity of GT development, computer models that predictively model sexual dimorphism of the GT may provide a useful resource to translate chemical-target bipartite networks and their developmental consequences across the human-relevant chemical universe. Here, we (i) construct a multicellular agent-based model (ABM) of GT development based on available biological information, (ii) evaluate the model's performance in recapitulating cellular changes underlying urethral tube closure, and (iii) use the model to examine how the interplay between SHH, FGF10, and androgen signaling is disrupted in urethral closure defects. The ABM simulates the actions and interactions of autonomous agents (cells) in a shared environment to assess their quantitative effects on the system as a whole. These models can distinguish key events leading to structural malformations, identify moments in development at which interventions have extreme consequences, and use systematic parameter sweeps to rank order system sensitivities [46–48]. The simulations make hypotheses and assumptions explicit for 'what-if' and 'what happens next' questions that can help inform predictive toxicology.

2. Methods and implementation

2.1. Scope of the GT model

A major challenge to systems-level simulation of GT development is defining an appropriately abstracted model and boundary conditions. One key genetic model for the study of biochemical signaling in GT development has been the mouse [10,16,32,35–38,49–52]. Gestational days E12.5 to E19.5 encompass the sequence of morphogenetic events from the urogenital sinus stage to formation of the urethral canal and distal opening. These events fashion GT development anatomically by temporal and spatial (proximo-distal) progression of cellular changes that are sensitive to fetal testosterone production, commencing at E15.5 after the onset of steroidogenesis during the Male Programming Window (MPW) from E14.5 to E.16.5 (as extrapolated from E16.5 to E18.5 in rats [53,54]). The period from E12.5 to E19.5 also encompasses the window of vulnerability to both sexual dimorphism and hypospadias in this species. Within this window, environmental disruptions invoke a range of GT malformations and degrees of hypospadias depending upon when exposure occurs and by what extent urethral morphogenesis is disrupted [10]. By E17.5, the male urethral primordium has divided into a central urethral tube and residual ventral seam. Therefore, for this study we modeled mouse GT development over the critical four-day period of gestation (E13.5-E17.5) that encompasses three major events in GT development: formation of endodermal urethral tube during elongation of the urogenital sinus (E13.5); patterning of the preputial mesenchyme during elevation of the urethral folds (E15.5); and sexually dimorphic changes leading to urethral fusion/septation at the ventral midline in males (E17.5).

We modeled gestational days E13.5 to E17.5 using an idealized two-dimensional (2D) cross-section (with dorso-ventral and medio-lateral axes) that represents GT morphology midway along its proximo-distal axis [10,51,52]. As shown in Fig. 1, the initial rendering (E13.5) of the modeled 2D cross-section consists of an exterior ectodermal jacket, an interior bud-like hillock of mesenchymal cells and an endodermal sinus; the entire structure was surrounded by a fluid medium. Our previous systems toxicology model of male reproductive developmental toxicity [44] identified vascular/angiogeneic processes as one possible target for chemical disruption (albeit not hypospadias). In mice, hypospadias is often accompanied by gross enlargements of the GT vasculature and reductions in HOXA13-dependent expression of EphA6 and EphA7 in the GT vascular endothelia [55]. The current GT model could be thus expanded by incorporating ephrin-signaling and angiogenesis as a future goal.