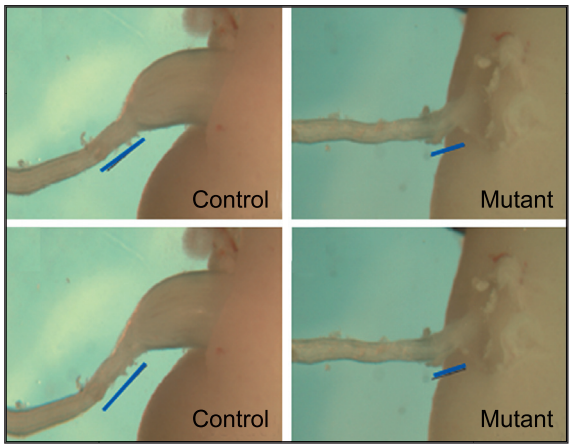
IV. Research Methodologies

20. Animal Models

Summary

At present, our understanding of cellular and molecular mechanisms of normal and abnormal development of the genitourinary system lags behind that of other organ systems. A broad program of research using model organisms is essential if this situation is to be remedied. While the mouse remains indispensable, non-mammalian models such as the zebrafish hold great promise; rat, chicken, frog, and other models will continue to have unique applications. Essential to progress will be collaborations; between clinician and basic researcher, between those working **in vivo** *and* **in vitro**, *and among those with expertise in different animal systems.*



Deletion of a calcium signaling protein, calcineurin, causes defects in pyeloureteral peristalsis, obstruction, and nephropathy in the developing mouse. Blue bars indicate length change in the most proximal segment of the ureter (from CP Chang et al. Calcineurin is required in urinary tract mesenchyme for the development of the pyeloureteral peristaltic machinery. *Journal Clinical Investigation* 113: 1051-1058 © 2004).

Basic understanding of development and maldevelopment of the GU tract will derive largely from work in model organisms. The mouse is the most widely used system for a number of good reasons: available resources and infrastructure include a sequenced genome, cDNA and genomic microarrays, tissue- and stage-specific cDNA libraries, and a multitude of mutant lines. Morphology and physiology is sufficiently similar to that of humans so that translational research can be accomplished. Among mammals, the mouse is uniquely amenable to intensive genetic and experimental manipulation. *In vitro* mammalian studies often use cells from rats as well as mice, and the rat is the primary model for physiology studies. Models for obstruction include sheep as well as rats, and bladder dysfunction has been studied mainly in cats and sheep.

Non-mammalian models have been underutilized but hold great promise for progress in this area. Zebrafish and medakafish have the advantage of being genetically tractable model systems with rapidly expanding genomic and proteomic resources and extremely short generation times. The power of the frog (*Xenopus*) system derives from the ease with which embryos can be manipulated. It is now possible to generate transgenic frogs, carry out genetic crosses, overexpress genes by electroporation and microinjection, and prevent gene function using antisense oligonucleotides or morpholinos. The

chick embryo has been a powerful system for developmental studies of numerous organs and, while not amenable to genetics, it offers a number of advantages over the mouse. Chick eggs are inexpensive, easy to obtain, and well suited to ***in ovo/vivo*** experimental manipulations, including tissue recombinations, surgeries, overexpression and knockdown of genes, and analysis of cell lineage by microinjection of lineage tracers and heterospecific transplantation. We suggest that a highly collaborative research effort continue across multiple experimental systems, profiting from the unique advantages and potentials of each.

Research Priorities

Multifaceted, collaborative approaches need to be strongly encouraged.

- Combine *in vitro* and *in vivo*/genetic approaches within the same animal model.
- Encourage collaborative efforts involving two or more investigators with expertise in different systems (e.g., *in vivo* versus *in vitro*, or in different animal models).
- Clinicians and basic researchers should work jointly to develop new *in vivo* and *in vitro* systems using model organisms.
- Use multiple animal models for the purpose of tissue engineering; this research will require interactions between cell and developmental biologists, clinicians, and physiologists.
- Investigate patterns of genitourinary malformations and disease in natural animal populations exposed to environmental contaminants.

Infrastructural Needs

All mutant mouse strains, particularly those produced in large centers, should be included in a database and be freely available for use as animal models or for *in vitro* studies of genitourinary disease.

21. Biomechanics

Summary

While biomechanics is a discipline essential to a functional understanding (from the cellular to the organ level) of organs of the genitourinary tract and to such goals as reengineering of the bladder wall, its application to urologic research has been limited. We list some important research goals in this area and underline the need to bring together selected specialists in biomechanical science and urologists with interests in the role of mechanical properties in urological disease.

Overview of Biomechanical Science as Related to the Bladder

Biomechanics, the study of mechanical forces in living cells and tissues, pertains to the bladder as a smooth muscle organ with dynamic prerequisites: urine storage at low pressure, and efficient and complete evacuation of urine through active muscle contraction. A comprehensive biomechanical description of the bladder would encompass the following topics, among others:

- fluid dynamics in non-rigid conduits including the bladder outlet, sphincter mechanisms, and urethra
- the mechanics of the individual cells (e.g., the effect of mechanical forces on the bladder smooth muscle cells, urothelial cells, and other cells in bladder)
- cell forces that may influence the extracellular matrix
- the assembly of connective proteins and other molecules that underlie the bladder's viscoelastic properties
- modification of intrinsic cell biomechanical behavior (e.g., muscle cell contractility and

blood vessel mechanics) by neurotransmitters and hormones

- the effect of mechanical bladder smooth muscle cell (BSMC) properties on cell phenotype and the surrounding microenvironment

Multiple cellular and molecular effects of mechanical stimuli have been described in whole animal bladder models, urothelium, BSMC cells subjected to strain, stretch, and compression, as well as the whole bladder in tissue culture. However, no systematic approaches have been established for such studies. Therefore, a more robust utilization of the principles of biomechanics, which can provide a conceptual framework to integrate mechanical force information from the molecular to the organ level, is clearly needed.

Biomechanics and Clinical Needs

If clinical practice is to be successful in maintaining the low-pressure storage and event-free evacuation critical to protecting the upper urinary tract, it must be informed by an understanding of the biomechanical properties of the bladder and its cellular and extracellular matrix components. It should be noted that biomechanical properties may also impact non-storage and emptying conditions, such as infection and tumor growth, in so far as microorganisms and tumor cells may respond to transient or permanent changes in cell biomechanics.

Biomechanics is of course fundamental to the long-term engineering goal of duplication of native bladder wall; here there is a need to establish minimal functional prerequisites necessary to guide design of functional bladder replacements. Also critical to the success of the bioengineering enterprise are models of long-term remodeling and engineered tissue survival that minimize the need for costly animal trials; because of the complexity of the remodeling process, *in vitro* model development should be undertaken first to avoid costly *in vivo* testing and development.

Research Obstacles

Our knowledge of bladder structure and shape—thought to be closely associated with the development and spatial distribution of wall tension—is very incomplete. Areas of contact with other pelvic structures still need to identified, quantified, and mapped out over a reconstructed, three-dimensional surface. We also know little or nothing about how the bladder layers are connected, to say nothing of the mechanical properties of these connections or how the bladder can accommodate large volume variations. Furthermore, a systematic approach to the *biomechanical* properties of cells with respect to their impact on bladder function and development is lacking.

Progress in these areas is greatly hindered by insufficient communication between clinical urologists and urologic investigators on the one hand, and biomechanical scientists on the other: clinical urologists and urologic investigators often possess only *ad hoc* knowledge of biomechanical science and do not appreciate its complexity; basic biomechanical scientists are largely unaware of bladder research goals and have not shown interest in developing clinically relevant models of bladder function.

Research Priorities

- Create new biomechanical models for the bladder with potential application to other systems (particularly the cardiovascular system). These should include organ culture models, and mechanical models describing tissue and cell behavior. Mechanical models of bladder wall function and wall stress should take into account the influence of adjoining pelvic cavity structures.

- Characterize the bladder's shape; areas of contact with other pelvic structures should be identified and quantified.
- Investigate the mechanical role of individual tissue components.
- Characterize the three-dimensional tissue structure of the bladder and other urologic organs from the cellular to the tissue level (1 – 1,000 μm)[1]

Infrastructural Needs

- Create a database of transgenic animals to collect unappreciated, non-utilized bladder tissues from *existing* transgenic animal models. This issue potentially cuts across all areas of bladder research.
- Convene a meeting/roundtable of selected specialists in biomechanical science and urologists with interest in the role of mechanical properties in bladder disease. The overall goal should be better communication of bladder biomechanical/biological requirements and questions to basic physical science, engineering, and biomechanics scientists.

[1] This will require high-throughput approaches to streamline integration of diverse imaging data into a three-dimensional structure. The structural information should be converted into databases suitable for immediate use in bioinformatic and computational biomechanics applications.

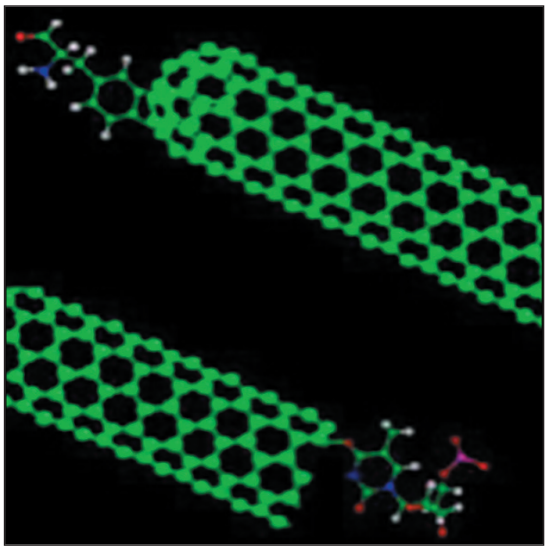
23. Nanotechnology

Summary

Nanotechnology is a new cross-disciplinary field emerging from technological advances that permit the manipulation of individual atoms and molecules. It promises to have manifold applications in disease detection, medical imaging, construction of complex biomaterials, and drug delivery and targeting. We propose steps to foster the active collaboration and communication between diverse scientific, engineering, and medical research communities necessary to develop the technologies that could revolutionize medical practice.

Nanotechnology has the potential to revolutionize biology and medicine by offering the ability to control and manipulate matter at a resolution that is at the molecular and atomic scale, in the 1 to 100 nanometer range. This means that scientists in this field are able work with individual atoms and molecules—a nanometer, or a billionth of a meter, is, about one eighty-thousandth of the diameter of a human hair, and only 10 times the diameter of the smallest atom. This technology does not derive from a single scientific discipline. Rather, it is a multidisciplinary combination of physics, chemistry, biomechanics, material science, and biology. Broadly speaking, nanotechnology incorporates:

- Research and technology in the length scale of approximately 1 to 100 nanometers
- Creation and utilization of structures, devices, and systems that have novel properties and functions because of their small and/or intermediate size
- Creation and utilization of materials, devices, and systems by building from the level of atoms and molecules



The electric properties of derivatized carbon nanotubes could make them invaluable as biosensors.

Two approaches to nanotechnology development are used: "top-down" and "bottom-up." In the top-down approach, development is driven mainly from the traditional disciplines of engineering and physics, and it makes use of current technologies like lithography used in making computer chips. This approach has the advantage of large-scale fabrication and more rapid integration of existing technologies. The bottom-up approach, starting from single atoms and molecules and exploiting the novel physical properties of the nanostructures, has more in common with chemistry and molecular biology. It uses the self-assembly of atoms or molecules to form nanometer-sized structures and is generally more difficult to control and characterize. However, in this field, both these approaches are often required concurrently. For example, in developing an antigen-specific sensor, the supporting circuitry would be fabricated from the top-down approach on traditional substrates, while the actual sensing element, consisting of biological molecules or nanoparticles, would be self-assembled.

Nanotechnology promises to allow us to communicate chemical information at a cellular and subcellular level, to place artificial components and assemblies inside cells, and to fabricate materials using self-assembly methods. New materials for use in pediatric urology could be created to act as templates for cell growth, structural components in tissue engineering, and antimicrobial surfaces. Nanostructured materials will facilitate early detection of diseases such as cancer and enable unprecedented advances in drug delivery technology.

As with any new field, applying breakthroughs in basic research in nanoscience to medicine presents obstacles to be overcome. Often it is difficult to fully characterize the structure or morphology of nanomaterials, particularly when they are functionalized or encapsulated. Typically, several advanced microscopy and analytical techniques such as NMR, EPR, and optical spectroscopy are required. Nanoparticles easily form aggregates, reducing their effectiveness as therapeutic agents, and toxicity studies have not kept pace with development and will lag for some time due to the proliferation of new nanomaterials.

Research Goals and Opportunities

It is difficult to encapsulate the research program of this new and diverse field in terms of priorities; we highlight some general directions for research that are likely to impact pediatric urology.

- **Develop biocompatible exogenous nanomaterials to control infection** and mitigate biofilm formation on the surface of long-term indwelling catheters, dressings, and engineered urological implants. Nanoparticles have been shown to be effective antimicrobials against bacteria and have shown the ability to penetrate biofilms.
- **Incorporate nanomaterials in tissue engineering.** Prosthetic bladders and urethra may benefit by the use of nanomaterials in the scaffolding to promote cell growth, quantify remodeling rates, and allow localized drug delivery. Nanomaterials might also be used

as templates for neuron growth when treating urologic diseases caused by spinal cord injuries.

- **Develop nanotechnology to improve drug targeting and specificity and allow timed, local release of drugs.** Tumor-restricted surface antigens may be targeted by adding specific nanoreceptors on the surface of nanoparticles. Drugs may be incorporated into nanoscale particles with coatings several nanometers in thickness, permitting controlled, local release of a drug through biodegradation of the encapsulant or stimulated release by radiation.
- **Create specific and rapid nanosensors for early detection of urological diseases using the properties of nanotubes and other technology.**
- **Enhance imaging systems for contrast at the cellular level using nanotechnology.** Nano-iron, and gadolinium particles can be used to enhance contrast in magnetic resonance imaging (MRI). Quantum dots allow visible and infrared imaging enhancement of tumors and cellular processes, and monitoring of tissue scaffolds during remodeling.

Infrastructural Needs

One of the most significant hurdles the new field presents is in education. Although the number of institutions offering programs in nanotechnology is growing, programs are primarily at the undergraduate level. Instruction needs to be developed for medical graduate and postgraduate students in pediatric urology to help them integrate the new technology. Since nanotechnology cuts across traditional disciplines such as biology, chemistry, engineering, physics, and material science, a new paradigm will be needed to train basic and clinical researchers.

Nanoscientists have materials and technologies in need of applications, and clinicians have problems in need of materials. A dialogue must be fostered between the two groups through seminars and workshops. Industrial, as well as academic, participation should be encouraged for effective cross-fertilization of ideas.

- Arrange joint mentoring of researchers by mentors with extensive experience in pediatric urology and nanotechnology.
- Fund new investigators taking a multidisciplinary approach to urology and nanotechnology.
- Encourage multidisciplinary research efforts between nanoscientists, urologists, and industry.
- Establish regional technical centers for nanotechnology and pediatric urology to assist with fabrication of new materials and their characterization.
- Develop educational programs to acquaint the next generation of urological researchers with nanotechnology.
- Convene workshops to foster interaction between clinicians and nanoscientists.

24. Stem Cell Biology

Summary

If the promise of stem cell research is to be realized in terms of beneficial treatments for patients, some major hurdles must be overcome. Methods for identifying, isolating, and enriching human stem cells—including those of the GU tract—at different stages of differentiation will need to be developed, and scientists need better means of tracking the location and fate of stem cells in the body. Much remains to be learned about the molecular signaling mechanisms that would mediate functional integration into human tissue.

Stem cells are undifferentiated, self-renewing cells that have the unique potential to produce many kinds of cells in the body. Various types of these cells are thought to be actively maintained in diverse adult tissues, including the intestinal lining, the hematopoietic system, and muscle. Their normal role in human disease is currently unclear. When grown in culture, stem cells can be induced to differentiate into many specialized cell types that form muscle, nerves, cartilage, blood, and other tissue. This property could revolutionize medicine, enabling doctors to repair specific tissues or to grow organs. A vast research effort has been directed to transplantation of these cells into animals with damaged or diseased organs—some improved function has been reported, but how enhanced function occurs and whether it derives from the function of differentiated stem cells remain unclear.

Major Research Obstacles

Significant challenges have to be overcome before any type of stem cell can be harnessed and translated to meaningful treatments for patients. These include identifying the optimal type of stem cell for specific assays and therapies for individual disorders, harvesting and growing sufficient quantities of the appropriate cell type, deciding the best therapeutic strategy for each condition to be treated, and assessing the potential side effects that may arise when such pluripotent cells are introduced into a patient. While methods exist to follow how some stem cells differentiate in animal models, there are no well-defined, non-invasive methods with which to study the survival, migration, fate, and function of stem cells in the living animal or human, and there are currently few markers, antibodies, or probes with which to distinguish specific classes of stem cell. Currently, little is known about the host environments that facilitate regeneration of tissue or the molecular signals that are responsible for tissue organization.

Research Priorities

- Isolate and characterize in molecular terms the stem cells from the GU tract.
- Investigate the effect of urological diseases on the fate of stem cells from the GU tract.
- Define *in vitro* culture conditions for maintenance and differentiation of GU tract stem cells.
- Use existing *in vitro* systems for complex morphogenesis to study cell biology, differentiation, and morphogenetic behavior of stem cells in three-dimensional environments.
- Utilize stem cells for drug discovery studies.
- Develop methods for identifying, isolating, and enriching human stem cell populations at different stages of differentiation.
- Develop reliable protocols for expansion, maintenance, verification, preservation, storage, and shipment of stem cells.
- Develop non-invasive methods and agents with which to visualize or track stem cells in the body.
- Develop therapies based on stem cells to treat urological diseases.

Infrastructural Needs

Advancing stem cell research will require dissemination of technical knowledge and skills in cell culture techniques across a variety of disciplines and disease research areas. There are few investigators who have sufficient experience with this research tool.

- Educational courses focused on the cell culture techniques for human embryonic stem cells; designated workshops for the dissemination of technical knowledge pertaining to isolation, characterization, maintenance, *in vitro* assessment, and *in vivo* application of stem cells.
- Standardized laboratory practices for use of human stem cells including proper exposure precautions, safe methods of disposal, and recordkeeping.
- Centers that will bring together basic stem cell biologists, researchers skilled in novel modes of cell delivery, urologists with disease-specific expertise, and investigators experienced in developing and assessing animal models of human diseases to create new research teams.
- A national stem cell repository and stem cell technology and bioinformatics core. This clearinghouse could receive, process, store, and distribute information on stem cells.

25. Clinical and Developmental Imaging

Summary

Imaging technology is central to both basic and clinical research in pediatric urology. In the area of basic research, understanding the genetic basis of genitourinary tract development depends on precise images of the developing urinary tract as researchers assess the consequences of a specific mutation or use gene fusions to localize specific proteins. On the clinical side, the key impact of imaging technologies is to provide a noninvasive means for researching the onset and extent of pediatric urology disorders, as well as to provide the means to evaluate effectiveness of treatment. The use of imaging technologies, such as prenatal MRI to examine genitourinary tract development in real-time, will provide, for the first time, the capability to assess the cellular and molecular processes affected during urologic malformation. This will facilitate the accurate diagnosis and treatment of common pediatric urologic disorders.

Research Priorities

Imaging innovations have progressed rapidly, but a rigorous and systematic assessment of the emergent technologies has not been performed. Formal assessments are required to determine whether they will actually result in improvements in GU-specific health outcomes and be cost effective.

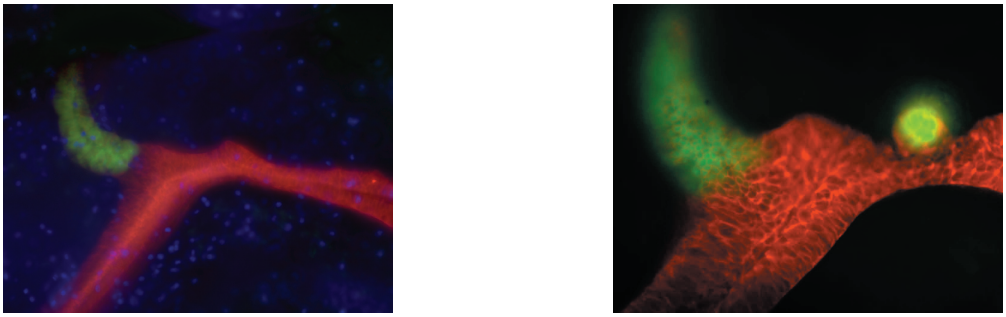
- Develop non-invasive imaging technologies:
  - to target GU abnormalities
  - to facilitate the analysis of prenatal GU tissues and improve the understanding of how congenital urologic malformations occur

  - to eliminate or reduce the radiation exposure to children during the diagnosis and management of common pediatric urologic disorders
- Develop imaging technologies to examine newly identified GU genes for their specific patterns of expression and function in tissues commonly affected by urologic malformations.
- Support the production of novel mouse lines bearing gene fusions and other constructs to facilitate the imaging analysis of gene function during prenatal GU development.
- Develop non-invasive imaging technologies for large animal models to identify processes and mechanisms involved in the diagnosis and therapy of pediatric urologic disorders.
- Examine whether the fusion of functional and anatomic images can replace invasive imaging procedures.
- Expand the use of low-radiation dose fluoroscopy and nuclear medicine technologies to facilitate the analysis of urologic disorders.

Infrastructural Needs

Pediatric urology requires a focused funding initiative to help insure that both the clinical and developmental imaging sciences are integral to the training and practice of pediatric urologists. Programs that provide enhanced research opportunities for pediatric urologists to understand imaging technology assessment and gene function in a developmental context will augment their clinical pediatric urology training and their effectiveness as caregivers.

- Establish programs in which education in imaging science is integral to the training of urologists.
- A platform for the sharing of imaging information on the developing urinary tract by clinicians and researchers



A mouse strain expressing a Hoxb7-Gfp transgene which is localized in the ureter and Wolffian ducts has been-stained with E-cadherin, a general epithelial marker that also labels the urogenital sinus, the primordium of the bladder, and urethra. The ureter and the Wolffian duct (both in green) are initially fused (left panel). During days 13-14 the ureter separates from the Wolffian duct, inserts in the urogenital sinus and is transposed to a new position at the base of the developing bladder (right panel). Disruption of this process underlies a number of developmental anomalies in humans including VUR, thought to affect 1 to 2 percent of the population (courtesy of Dr. Catherine Mendelsohn, Columbia University).

26. Systems Biology

Summary

Technological advances in the separation and characterization of biomolecules, coupled with the emerging field of bioinformatics, have allowed for the acquisition and organization of enormous amounts of data describing an organism in terms of gene and protein expression, metabolism, DNA modification, and other parameters. The possibilities for identifying relevant biomarkers to assist the clinician in the diagnosis and treatment of specific diseases are enormous. We propose some specific steps to ensure that clinical investigators in urology can take advantage of this new field.

"Systems Biology" is a new term for the discipline forming in response to the assembly of enormous data sets of biological information, data sets made possible by advances in biotechnology—automated DNA sequencers, chip-based methods of transcriptome and genome scanning, mass spectrometry-based methods for sequencing proteins, and many others. These data sets are quite diverse; they might include the sequences of entire genomes of related organisms, tables specifying the relative expression of all the genes in an organism in a particular tissue under specific conditions, or the relative amounts of all different proteins in a particular cell.

The organization and analysis of the data to address meaningful questions in biology and medicine depend on powerful computational tools and the emerging discipline of bioinformatics. The transfer of data between laboratories, across technical platforms, and between investigators with divergent expertise presents complex problems whose solutions are now in their infancy, and are a focus of active research.

The categories below give some idea of the breadth of investigations in systems biology:

Genomics

The systematic study of the genetic information of an organism (genome) offers researchers the prospect of developing new insights into the genetic basis of disease. Based on assays for single nucleotide polymorphisms (SNPs) in populations, scientists can identify individuals who are at genetic risk for particular diseases or particular adverse outcomes in the clinic. Studies often extend beyond one generation and require tracking of ancestral groups and migration/immigration patterns, and the number of specimens and the genetic loci to be analyzed in these studies is enormous. Additional challenges occur at the level of computation and data analysis. Several high-throughput platforms are becoming available for rapidly assessing SNPs, permitting us to envision a genetics-based urologic practice.

Transcriptomics

With the advent of high-density oligonucleotide or cDNA arrays, the transcription of thousands of genes in a particular biological sample can be studied simultaneously. After a decade of efforts toward standardization of array platforms, attaining accurate annotation of thousands of genes, and development of data-mining software, global gene profiling has becoming a common experimental tool. If its application in pediatric urology is still limited, the conceptual and technical foundations now exist for: (1) comprehensive description of transcriptomes (the set of all RNAs in an organism) and deeper insight into the program of transcriptional regulation; (2) identification of novel molecular pathways for the discernment of new disease mechanisms; (3) discovery of gene clusters whose expression are altered in a pre-disease state (biomarkers) or are associated with subsets of patients (patient-stratification tools), and; (4) identification of molecular targets of

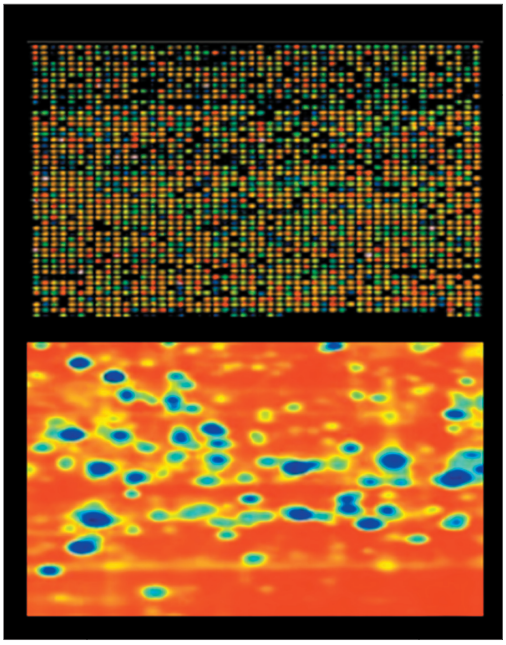
value to intervention and classification of patients' responses. When combined with appropriate animal models or well-designed clinical trials, this technology may become one of the most powerful tools for pediatric urologic investigations.

Epigenomics

Epigenetic mechanisms mediate heritable changes in gene expression that do not involve a change in DNA sequence *per se*. They can involve either chemical modifications of the DNA itself or modifications of proteins that are closely associated with DNA. Epigenetic regulation controls important biological processes such as imprinting, X chromosome inactivation, and transcriptional control of gene expression; its disruption contributes significantly to the etiology of many human diseases. In the past decade, "epigenomics," or the study of whole-genome epigenetic changes, has gained momentum with the advent of modern investigative tools: methylation-sensitive fingerprinting, restriction landmark genome scanning, and methylation-specific oligonucleotide arrays that allow identification of aberrant hypo- or hyper-methylation sequences and simultaneous assessment of methylation status of multiple CpG islands. Like the SNP- based approaches for the systematic analysis of sequence, determining the methylation patterns of a large enough number of genes has the potential to yield highly discriminating signatures for specific diseases.

Proteomics

The field of proteomics began with efforts to determine the structure, expression, localization, activity, and cellular roles of the ensemble of proteins (proteome) in the body. Great progress has been made in this field of research owing to novel instrumentation, experimental strategies, and bioinformatics methods. A more contemporary goal of proteomics research is to obtain a comprehensive, quantitative description of expression of hundreds, if not thousands,



Two methodologies that permit a systems biology approach. In gene expression arrays (top), the immobilization of defined DNA sequences at particular points in a grid allows for simultaneous measurement of the abundance (reflected by pixel color) of all RNAs in a biological sample. In the lower panel, the electrophoretic separation of a complex mixture of proteins allows the amount of each protein component to be quantified. In both approaches, sophisticated methods of data acquistion and analysis allow clinicians to identify patterns of abundance that are markers for diseases.

of proteins/peptides, and changes in their interrelationship under the influence of biological perturbations such as disease or drug treatment. The complex information that proteomics provides has the same potential for providing biomarkers and other predictive information as the gene-based approaches outlined above. Current state-of-the-art methodologies of proteomics permit (1) pattern recognition (without identification of specific proteins/peptides) via protein profiling by sophisticated separation methods; (2) identification of proteins/peptides using a multidimensional protein identification technology (MudPIT); (3) relative and absolute protein quantification using techniques such as isotope-coded affinity tag (ICAT) labeling, iTRAQ, and metabolic labeling-

MS; and (4) construction of two-dimensional ion density maps of proteins directly from the surface of tissue sections using MALDI imaging.

Metabolomics

Metabolomics is the systematic study of the metabolites of a cell or organism. Traditionally, characterization of abnormal metabolites in blood, urine, or tissue biopsies has been used to detect diseases such as inborn errors of metabolism. However, the concept of parallel analyses of all the metabolites in a biological sample has been made possible only because of rapid advances in technologies such as gas chromatography mass spectroscopy. With these technologies, all the metabolites in the sample set are taken into statistical consideration to derive the algorithms permitting disease or disease-stage identification and prediction of patient response.

A major goal of systems biologists is the creation of "networks" of various kinds. These include signal transduction, protein-protein, DNA-protein, and three-dimensional mapping types of networks. For example, one extension of traditional proteomic approaches would be to characterize, using yeast two-hybrid methods, the set of protein-protein interactions that characterize the state of a living cell.

Relation to Clinical Needs

The value of the systematic approaches briefly described above might be illustrated by recalling traditional approaches. Urinary markers of VUR, for example, have been identified using educated guesses as to what markers might be important, and testing them with relatively crude detection technologies such as Western blotting. In the systemic approach, all of the proteins that appeared in the urine during the course of VUR (and during its spontaneous resolution, and in uninfected patients) can be systematically identified and, using computer-based analytical techniques, patterns that are specific to VUR can be ascertained. With the advent of proteomics, genomics, and epigenomics, we are able to identify many more potential biomarkers or highly discriminative marker-clusters that may serve as diagnostic, prognostic, and therapeutic tools. Significant challenges remain in identifying sufficient numbers of these markers or marker-signatures, testing them through case-control studies and prospective trials, and developing the statistical and computational tools necessary to analyze and model complex data sets.

Research Opportunities

Most major teaching centers include facilities and expertise for genomics research. Proteomics capabilities also are becoming more common, and within a few years will be ubiquitous at major centers. The proximity of these technologies and specialists trained in their use means that it will be possible for clinical and basic scientists conducting urologic disease research to use these approaches in their own studies. Some specific research opportunities are:

- Establishment of databases of gene and protein expression patterns in the genitourinary tract, at various stages of development in health and disease.
- Application of urine proteomic information to developing biomarkers of diagnostic and predictive value, including markers that predict response to certain clinical interventions.
- Identification of at-risk populations based on systems biology approaches.
- Identification of markers for cell types destined to become components of the upper or lower collecting system.
- Creation of cross-platform approaches that incorporate relevant animal models.
- Use of pharmacogenomics to discover new drugs and treatment protocols.

- Identification of maternal factors that contribute to pediatric disease.
- Decoding gene function and complex biological pathways that govern diseases through the identification of unique transcriptomes and their master switches.

Infrastructural Needs

There is a significant technical, cultural, and conceptual chasm between most clinical and basic researchers in urology and those doing genomics, proteomics, and other systems biology studies. Most systems biology experts have little familiarity with urology as a discipline, and often little or no familiarity with medical fields in general. A common misconception of basic researchers in "leading edge" fields is that urology-focused questions are either trivial, derivative, or otherwise uninteresting. Because it is more likely that long-term interest in an area will be spawned when one "grows up" in it, pediatric urology needs new mechanisms for attracting gifted and promising graduate students and junior scientists with an interest in basic research. Research training programs specifically focused on pediatric urology, and in urologic disease in general, need to be developed and implemented.

Systems biology is a technology-driven area. Consequently, infrastructure needs are great and require an extremely large amount of financial capital and other resources. Leaders of urology research programs need to be positioned within their own institutions in a manner that will allow them to maximize their colleagues' access to and participation in the newly forming infrastructure elements (e.g., genomics programs and proteomics core facilities) that are relevant to systems biology studies.

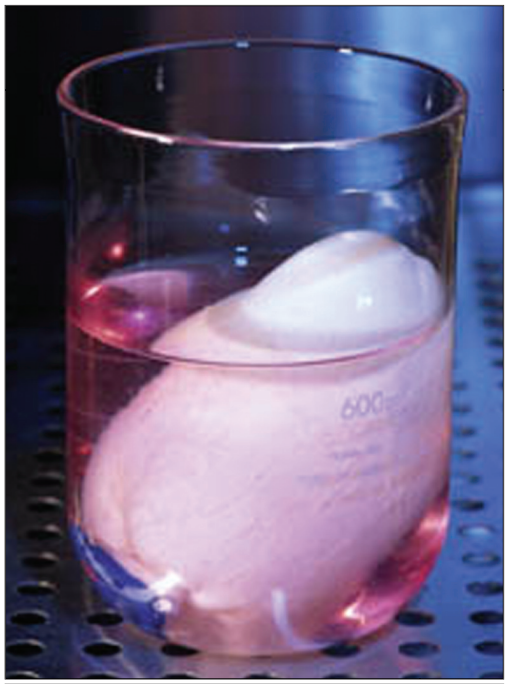
- New NIH pre- and postdoctoral training programs integrating systems biology with urological disease research.
- Better participation of national medical organizations (the American Urological Association in particular) in maintenance and development of academic training programs that promote state-of-the-art research involving clinicians.
- Systems biology requires high levels of dedication and willingness to acquire new expertise. Integration of systems biology research with urology will require clinicians to focus less on high-volume patient care and make more of a commitment to research.
- Development of financial instruments within academic urology practices that will allow research-oriented urologists to pursue academic research careers without being overwhelmed with clinical demands.
- Establishment of accessible tissue banks linked to comprehensive clinical databases.

27. Tissue Engineering

Summary

Millions of American children suffer from congenital and acquired urologic diseases. Dysfunctional organs from these diseases interfere with the normal development of the child and have a large economic impact on our health care system. In the future, tissue engineering will play an important role in the treatment of pediatric urologic disease. Recommendations for future research on tissue engineering include:

- *Conduct tissue engineering research of the bladder, urethra, kidney, and pelvic floor with the following goals:*
  - *Increased understanding of normal and abnormal cells and developmental biology*
  - *Development of cell therapy*
  - *Development of tissue and organ replacement*
- *Enhance training and education of physician/scientists in tissue engineering.*
- *Enhance interdisciplinary collaboration and education.*
- *Establish appropriate infrastructure, training, and core facilities for research.*



Engineered Bladder Prior to Implantation. A small piece of tissue is obtained from the bladder in need of replacement or repair. The progenitor muscle and urothelial cells are expanded and seeded separately on each side of a three dimensional bladder shaped scaffold. The construct is placed in a bioreactor where the tissue is allowed to grow. One week later, the engineered bladder is ready for implantation (courtesy of Dr. Anthony Atala, Wake Forest University).

Diseased pediatric urologic tissues have traditionally been replaced or repaired with autologous tissues harvested from other sites of the body or by heterologous transplantation. However, these approaches rarely replace the entire function of the original tissue. For example, many congenital and acquired pediatric urologic conditions render the child's bladder dysfunctional. When these dysfunctional bladders require reconstruction, a surgical procedure called bladder augmentation is required. This complicated surgical procedure, which uses tissue from the patient's own gastrointestinal tract, is associated with significant complications, including infection, intestinal obstruction, mucus production, electrolyte abnormalities, perforation, and cancer. Other pediatric urologic disease states—hypospadias, exstrophy, and epispadias, posterior urethral valves, prune belly syndrome, intersex, urinary incontinence vesicoureteral reflux, renal failure, and hormone-deficient states—require replacement of organs or organ systems, and the need for advances in tissue engineering to meet this need is critical. Tissue regeneration may or may not require cells. If cells are needed, several sources might be available, including mature stem progenitor stem cells and

other cell types occurring naturally in a person's body (autologous) or derived from another source (heterologous). Stem cell populations associated with the urinary tract recently have been identified, although their characterization is incomplete.

Current tissue engineering techniques using cell-scaffold composite grafts to induce regeneration have utilized cultured cells from the *normal* animal organs. However, future clinical application of this technology in patients with abnormal organs will involve the use of cells derived from the pathologic abnormal organs. For normal organ regeneration process to occur successfully in humans, cultured cells from the *abnormal* organs must not be terminally differentiated and must possess normal proliferative and functional characteristics, and differentiate normally. Unfortunately, little knowledge exists as to whether cells from an abnormal organ retain normal growth patterns and functional characteristics in an *in vitro* culture environment.

Priorities for Basic Research

During the past 2 decades, it has been established that the bladder, urethra, penis, vagina, ureter, pelvic floor, and kidney have the potential to regenerate. From this work, it is now clear that interactions between the epithelium and mesenchyme are essential for the formation of normal tissues and organs. These discoveries may be exploited soon to generate sizable quantities of tissue suitable for study and transplantation, provided that strategies can be developed to isolate, expand, and differentiate native, autologous cells *in vitro* and *in vivo*. This will require that cellular signaling or growth factors that mediate the development of normal functional tissues be identified.

- Elucidate the cell signaling that occurs between the epithelium and smooth muscle of pediatric urologic organs and the role of extrinsic growth factors and their receptors.
- Define further the immunobiology of biomaterials used for tissue engineering.
- Define the effects of biomaterials on surrounding normal tissue.
- Determine how the mechanism of tissue regeneration differs from normal wound healing.

Priorities for Translational Research

It is a reasonable prospect that advances in cell biology and cell research may enable scientists to grow tissues and organs in the laboratory—a small population of cells given the appropriate molecular cues would proliferate and differentiate into functional organs. This would eliminate many of the current obstacles to organ replacement, such as donor-recipient mismatches and limited organ availability.

- Develop models of long-term remodeling and engineered tissue survival to minimize costly animal trials.
- Explore the effect of the pediatric urologic organ biomechanical properties on early regeneration.
- Develop methods to manipulate the local environment (through the scaffold material, extracellular matrix, or appropriate growth factors) to induce the controlled regeneration of tissues or organs starting from these cells.
- Define and standardize biomaterials in terms of such properties as elasticity, induction of cellular proliferations, and stability.
- Explore the use of biomaterials with bioactive functional groups to increase revascularization and other processes critical to normal regeneration.
- Establish biomaterial preparation and scaffold production and standardization laboratories.
- Develop cell production capabilities and cellular function tools.

Infrastructural Needs

The development of tissue engineering technologies is currently hampered by a lack of appropriately trained investigators. The new technologies that would most likely yield the greatest impact are not taught well, and few training programs provide students with in-depth skills to perform tissue engineering research.

- Institute training awards that allow outstanding health scientists to develop expertise in tissue engineering and materials science under the guidance of outstanding investigators in this field.
- Establish Centers of Excellence dedicated to tissue engineering.
- Establish a kidney, bladder, urethral, and pelvic floor primary and stem cell bank that would include a depository and central distribution center for sharing cells, reagents, and model systems.
- Establish a patient registry to assist in obtaining needed samples from a large population.

Public interest and educational efforts

As the sciences of tissue engineering continue to be developed, scientists should not overlook particular experimental therapies that could raise ethical concerns among some members of the public. Forums that discuss the ethical, legal, and social issues in biomedical research should guide policy decisions in these areas. These forums include the NIH Office of Biotechnology Activities, the NIH Office of Human Research Protections, and the President's National Bioethics Advisory Committee

Ongoing communication among scientists, physicians, educators, ethicists, theologians, elected officials and the public is essential to guide the future of this research, and to ensure that America continues to invest judiciously and responsibly in biomedical research.

28. Clinical Trials and Epidemiology

Summary

Pediatric urologic practice should be guided by the kinds of objective criteria that can be only obtained through sound clinical trials and valid and comprehensive epidemiological studies. Top priorities are:

- ***Establishment of standard definitions of pediatric urologic conditions for use in clinical practice and research***
- ***Development of a set of standardized objective and patient-centered outcomes for use in clinical research of various pediatric urologic conditions***
- ***Organization of clinical research networks to undertake randomized clinical trials***
- ***Creation of pediatric urology disease registries for use in clinical research and improvement of quality of care***

Pediatric urology is a focused subspecialty of general urology that deals with relatively uncommon congenital disorders. Consequently, *clinical research is hampered by difficulty in assembling adequate samples from which to draw meaningful conclusions. The research enterprise also is hindered by a lack of a standard nomenclature and validated outcomes for various conditions, and the absence of a centralized support structure and inadequate funding.*

Current Needs in Clinical Research in Pediatric Urology

Standard definitions

There are no established standard classification systems or nomenclature for many pediatric urologic conditions (e.g., hydronephrosis, hypospadias). This is a significant hindrance to clinical research, as existing studies often focus on disparate populations

of affected children with the same condition, limiting the generalizability and validity of the results. *A series of NIH-sponsored multidisciplinary workshops should be convened with the explicit goal of developing these definitions and classification systems*. Each workshop would address a specific pediatric urologic condition. Patient advocacy groups and professional societies would be included in the planning process and the workshop itself.

Training in clinical research methodologies

There are few investigators in pediatric urology formally trained in clinical research methodologies such as clinical trial design, epidemiology, or health services research. Targeted NIH funding for new clinical researchers in pediatric urology is recommended. Included in this initiative should be health care professionals from other disciplines—behavioral specialists, social scientists, and others—to promote an interdisciplinary model of care for patients with urological conditions and an associated research agenda. Workshops on topics ranging from study design to grant writing should be held to encourage the development of advanced research skills and promote new collaborations among investigators.

A central coordinating center

Pediatric urology clinical researchers do not have adequate support services to generate pilot data with which to support initial grant applications. *We suggest the establishment of a central clinical research and epidemiology coordinating center with expertise in biostatistics and study design to support novel studies in the field*. This shared resource would then be available to support new investigators and assist in generating pilot data for future grant applications.

Widely accessible, standardized databases to support clinical research

Currently, there is no agreement on the required data elements for databases of pediatric urologic conditions, and there are significant differences in the information technology platforms of existing databases that prevent data sharing. *Shared databases with common data elements should be developed, with the software for these databases available to all investigators*. Software should be constructed in such a manner that participating centers have the option of uploading data (stripped of personally identifying information) to the World Wide Web for analysis in the aggregate. As new data platforms are developed, they should be vetted by NIDDK staff, representatives of the various professional societies, and patient advocacy groups to ensure both the clinical utility and research applicability of the software. If feasible, a standing board should be created to facilitate this process.

Clinical Research Networks

There are no established clinical research networks in pediatric urology and few adequately sized randomized clinical trials have been completed. Collaborative networks will be needed for the conduct of large, randomized clinical trials. These networks should include a mix of academic and community medical centers, and they should utilize an interdisciplinary approach.

Disease registries

Patient registries, essentially databases containing information about clinical characteristics (e.g., gender, classification of condition, description of treatment), treatment interventions, and outcomes of a patient population with a particular condition, are vital tools in clinical research. Particularly useful in conditions that are relatively rare, registries can merge a geographically diverse patient population and thereby aid the development of randomized clinical trials and observational studies. There are few adequate disease registries available to address scientific questions in pediatric urology. Those that do exist tend to be from single institutions and are either not generalizable or of inadequate size. *We propose the funding of patient registries in pediatric urology that share the following*

features: (1) standardized collection of patient demographic information (including socioeconomic status, race/ethnicity and other sociodemographic factors); (2) standardized nomenclature of disease and descriptions of treatments, procedures, and outcomes; (3) inclusion of health-related quality of life (HRQOL) measures; (4) methods to ensure the generalizability of the collected data; and (5) collection of serum and tissue for deposit into existing NIDDK repositories.

Standardized objective and patient-centered outcomes

There is great debate regarding which objective endpoints should be used to define a successful outcome after treatment for many conditions in pediatric urology and, at the same time, there are few patient-centered tools for assessing outcomes in these diseases. **We suggest** *that standardized objective outcomes (short- and long-term) must be developed for use in clinical research in pediatric urology*. Included in these should be functional status and patient-reported symptom severity and HRQOL. The opinions of patient advocacy groups should be solicited in the development of these measures. Disease-specific HRQOL instruments should capture the distinct perspectives of the patient, his/her parents, and the health care provider. New instruments should provide a unique and much-needed perspective on patients' physical and psychosocial outcomes that are less focused on "pathology" (e.g., clinical depression) and more focused on adjustment to daily living. Finally, these instruments should be culturally sensitive and should be validated in Spanish and well as English, in an effort to capture the unique impact of pediatric urologic disease in minority and underserved populations.

Inclusion of minority and underserved populations

Many clinical studies do not enroll adequate numbers of minority or underserved subjects, limiting the generalizability of the results. Recent evidence suggests that careful preparation and planning of clinical studies can significantly increase minority and underserved subject recruitment and retention. Two models have been developed to guide the investigator: the Interactional Model for Recruiting Ethnically Diverse Research Participants and the Recruitment Triangle. These models essentially tailor the study personnel and recruitment strategies to the target populations, identifying key barriers to recruitment of minority and underserved individuals, and developing successful strategies based on incentives, outreach to parents and health care providers, improved communication, and other elements. They should be used in pediatric urology clinical research.

Identification of risk factors

Few genetic, environmental, and behavioral risk factors for the development of pediatric urologic conditions have been identified. Standard epidemiologic research methodologies, such as case-control and observational cohort designs, should be employed to identify genetic, environmental, and behavioral risk factors for pediatric urologic disease. Birth certificate registries, like the one in the State of Washington, should be utilized to help identify cohorts as well as *in utero* exposures that put children at risk for development of pediatric urologic disease.

Top Priorties

- Establish standard definitions of pediatric urologic conditions for use in clinical practice and research.
- Develop a set of standardized objective and patient-centered outcomes for use in clinical research of various pediatric urologic conditions.
- Establish clinical research networks to undertake randomized clinical trials.
- Create pediatric urology disease registries for

use in clinical research and improvement of quality of care.

Additional Priorities

- Foster the development of clinical research within pediatric urology by establishing a central clinical research and epidemiology coordinating center to support novel studies in the field.
- Increase the number of pediatric urology investigators formally trained in clinical research methodologies.
- Develop bioinformatic software for use in pediatric urology and make it available in the public domain for use by all interested investigators.
- Encourage the enrollment of minority and underserved populations into pediatric urologic studies.
- Identify risk factors for the development of and/or severity of pediatric urologic diseases.

29. Therapies and Diagnostics

Summary

Research that seeks to rigorously assess the usefulness of new technologies in pediatric urology, with the ultimate emphasis on child health outcomes, as well as cost-effectiveness, is strongly encouraged. Collaboration between industry and academia is essential for the development of new diagnostic and therapeutic technologies; professional societies can play a vital role in developing evidence-based guidelines for their use, and modern methods of communication by the National Institutes of Health and other organizations can ensure that the public and health care professionals have the information they need to guide them.

Many new technologies (e.g., endoscopic correction of vesicoureteral reflux) come into common clinical practice before a complete evaluation of their societal value or cost-effectiveness has been documented. In some instances, the efficacy of these new technologies can be questioned based on the inadequacy of the original clinical study design. However, once these technologies become commonplace and widely used, it is difficult to re-evaluate their role and change clinical practice patterns. *This mandates that a more careful assessment of new technologies be performed prior to widespread implementation.*

Assessment, Validation, and Diffusion of New Technologies

The critical assessment of a new pediatric urologic technology should be based on a well-validated conceptual framework. Thornbury and Fryback's "hierarchy of levels of efficacy" guides the logical evaluation of a new technology from the laboratory to clinical practice. Specifically, the framework directs the performance of studies in a defined order, and provides data on the technical feasibility (level 1), diagnostic accuracy (level 2), impact on diagnosis (level 3), changes in clinical management (level 4), improvement in child health outcomes (level 5), and societal value/cost-effectiveness (level 6). Special emphasis, especially in those diseases having long-term ramifications, should be placed on the last two levels, which are generally neglected in childhood diseases. Without long-term assessment with perpetual databases and ongoing statistical efficacy and cost analysis, the cost-benefit analysis that should be the basis of health care policy cannot be performed.

Workshops and forums to discuss ethical, legal, and social issues related to the new technology, and best practice adoption through evidence-based guideline formation by professional organizations, also are needed. Modern information and communication technologies are effective tools to help in the

collection, processing, and targeted distribution of information from which clinicians, researchers, administrators, policymakers in health, and the public can benefit.

Corporations have the financial resources and the incentives to develop new technologies; academic medical centers provide the medical expertise and the infrastructure to evaluate and implement these new technologies. In spite of the mutual need, complex legal and financial issues have sometimes stymied corporate collaboration with academic research. This is particularly true in pediatrics, where pharmaceuticals and medical devices are not often developed or tested and where there are few incentives to include children when evaluating new technologies.

Priorities

- Support research proposals that incorporate rigorous technology assessment with emphasis on health outcomes and cost-effectiveness.
- Encourage development and testing of new technologies in children.
- Establish grants targeted at collaborative research in pediatric urology between industry and academic medical centers.
- Disseminate evidence-based information to the lay public and health care professionals.
- Partner with professional organizations to develop evidence-based guidelines.
- Encourage forums to examine ethical, legal, social issues related to new technology in children.