Research in Pediatric Surgery

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Abstract

Pediatric surgeons have the privilege to care for patients at every stage of human development, from the fetus to the fully developed young adult. As such, we must cultivate and advance an all-encompassing knowledge base ranging from obstetrics to pediatrics to adult medicine and surgery. This unique, sweeping perspective on human disease requires an equally broad approach to research, which in our field is as vast and varied as it is stimulating.

Keywords

Surgery research • Newborn surgery • Animal models • Regenerative medicine • Innovation

3.1 Introduction

Pediatric surgeons have the privilege to care for patients at every stage of human development, from the fetus to the fully developed young adult. As such, we must cultivate and advance an all-encompassing knowledge base ranging from obstetrics to pediatrics to adult medicine and surgery. This unique, sweeping perspective on human disease requires an equally broad approach to research, which in our field is as vast and varied as it is stimulating.

Yet, despite the appeal of research in such a diversified and vibrant spectrum, the relative proportion of pediatric surgeons performing research appears to have been dwindling in recent years. While different factors can be debated as implicated in this scenario, perhaps one should be emphasized, namely the increasingly restricted exposure to research during training. The greater significance of this trend lies in the fact that, unlike most other components of this conjuncture, it has career-long consequences, rendering the unexposed trainees essentially unable to develop as independent investigators once they become practicing pediatric surgeons, notwithstanding the eventual will to do so. Regrettably,

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fewer and fewer countries and institutions can afford the time and resources necessary for surgical trainees to develop a strong research background.

While the recent emphasis on a multidisciplinary approach to research has allowed for many developments that would not have been possible in isolation, we must aspire to a central role within research groups related to our specialty. Clinical expertise is often a pre-requisite for one to provide consequential guidance to the powerful scientific methodology currently available and the perspective offered by the pediatric surgeon cannot be replaced. By the same token, only we can protect and expand the role of research in the education of our future peers. This chapter is aimed at tendering some support, however limited, to this need.

It would be beyond the scope of any book chapter to present a comprehensive, exhaustive review of all the possible developments applicable to research in Pediatric Surgery. Here, we present a summarized overview of different aspects involving both laboratory- and clinical-based research that should be of interest to both trainees and practicing colleagues, through select examples representative of the far reach of our field. Our focus will be on translational research, as this is typically the chief dominion of the pediatric surgeon, as opposed to that of the basic scientist.

3.2 Animal Models

Although much can be learned from *in vitro* analyses of intracellular processes and defined cellular manipulations, especially in light of recent developments in cellular reprogramming, the complexity of organ systems or whole organisms cannot yet be substituted. Both developmental and interventional research pursuits still depend heavily on animal models, which remain the workhorses at most pediatric surgical laboratories.

There is now an overwhelming variety of animal models for research, spanning widely across taxonomic groups [1]. A few basic considerations should guide animal selection for a given experiment. One is of course the degree of correspondence to the human disease or biological process of interest. The options here are perhaps surprisingly broad, depending on the subject, not infrequently including significantly less prescient species, such as in the zebra fish model of lymphatic malformation, in addition to more predictable mammals. At the same time, species-specific variations in physiology and anatomy can render certain higher species essentially irrelevant to a given human disease process. For example, a swine model of naturally occurring congenital diaphragmatic hernia (CDH) (*Sus scrofa*) does not include pulmonary hypoplasia, virtually ubiquitous to CDH in human infants.

Another consideration is the availability of genetic tools conducive to in depth molecular and pathway-specific analyses of mechanisms behind the phenomena being studied. Mice (*Mus musculus*) constitute the prime representative of that set of considerations, not the least due to the plethora of knockout and knock-in murine models, though the thornier rat knockouts have also become options in the last several years. The International Mouse Phenotyping Consortium is striving to create viable strains of identical genetic background mice in which only one of the approximately 20,000 genes in the mouse genome can be selectively deactivated for systematic phenotypic screens, further expanding the scope of the murine genetic manipulation platform [2]. The more recent development of the first cloned rat also paves the way for the establishment of overexpression rat models based on targeted insertions [3].

Yet another consideration of special appeal to pediatric surgery is tolerance to fetal manipulation/intervention. While this can be accomplished in a number of species, sheep (*Ovis aries*) deserves special attention due to their inordinately high tolerance to such manipulations, the size of their fetuses and newborns, and the easily manageable gestational times. The ovine model can also be an asset to an additional aspect to be taken into consideration when selecting an animal model, namely is fast growth rate combined to the fact that their sizes are comparable to that of humans, from infancy to adulthood.

Meaningful growth is often a pre-requisite to pediatric surgical research, for example in projects involving different forms of structural repair.

Expectedly, as always, logistical and financial constraints come into play as well. The following is a brief review of select animal models of interest to certain specific groups of pediatric surgical diseases, as representative illustrations of the breadth of animal research in our field. Other lists equally focused on our specialty, though based on somewhat different criteria, should also be of particular interest to the reader [4].

3.2.1 Abdominal Wall Defects

Not infrequently, a given structural congenital anomaly can be modeled in animals by either of five methods: surgery; genetic manipulation; drugs/chemicals; other environmental manipulations; or it may be naturally occurring. Selecting which one best correlates with the clinical disease is not always straightforward, especially when the etiology of the human condition is unknown. Animal models of gastroschisis and omphalocele illustrate that scenario.

There is an inbred mouse strain, namely HLG/Zte, in which gastroschisis occurs spontaneously. The typical prevalence of 3% can be increased with irradiation during pre-implantation development [5]. Studies with these animals have identified a region of the mouse chromosome 7 as a responsible locus [6]. Further similar studies may shed more light on genes eventually involved in the development of abdominal wall defects.

Abdominal wall defects can also be induced experimentally with a variety of teratogens. However, these agents typically lead to inconsistent results, as well as multiple associated anomalies. Aminpyrine causes omphalocele when given to pregnant mice at midgestation [7]. This can be augmented by supplementation with barbital [8]. In rats, omphaloceles have been induced with maternal exposure to DA-125 (anthracycline antineoplastic agent) [9], beta-aminoprpioitrile [10], or flubendazole [11]. Additional teratogenic agents have been explored in assorted species, including doxorubicin hydrochloride [12], ethanol [13, 14], nitrous oxide [15], ethylene glycol [16], scopolamine hydrobromide [17], acetazolamide [18], and cyclooxygenase inhibitors [19]. The rate of gastroschisis in this studies, however, is somewhat limited, ranging from 3.7 to 19.8%. In guinea pigs, a daily period of maternal hyperthermia can result in abdominal wall defects, among other abnormalities [20].

Various species have been used in surgical models of gastroschisis, the most prominent of which are sheep, rabbit, and the chicken embryo. Haller et al. first described a sheep model of gastroschisis by operating on fetal lambs at midgestation and excising a full thickness disk of abdominal wall lateral to the umbilical cord [21]. The exposed intestine in surviving fetuses was edematous and matted, similar to the findings in humans. Langer and colleagues later modified this model by placing a silastic ring in the abdominal wall defect [22–24]. Though this design was associated with a relatively high rate of spontaneous abortion, it demonstrated that intestinal damage correlates with the time of exposure to the amniotic fluid. Less costly rabbit models of gastroschisis have also been described [25, 26]. Improvements in experimental fetal surgery have improved the success rate of this model to the range of 80–90% [27, 28]. An interesting modification of the leporine model has been described so as to remove the effect of amniotic fluid exposure on the intestinal damage during fetal development [29]. The least costly models for gastroschisis involve chicken embryos. The chicken embryo is enveloped in amniotic fluid and a number of membranes. After confirming fertility of an egg, a 1 cm defect is created in the shell. Using the allantoic vessels and the umbilical cord as landmarks, the physiologic umbilical hernia sac can be incised in order to create a gastroshisis [30]. Using this model, the effects of amniotic fluid exchange as a means to reduce the severity of intestinal damage have been studied [31–33].

3.2.2 Biliary Atresia

Multiple theories have been proposed to account for the varied spectrum of pathology in biliary

atresia, ranging from putative congenital malformations of the bile ducts to the presence of a causative infectious agent. Reflecting this diversity, several types of models have been described.

Lampreys provide an arguable natural model for biliary atresia. Adult lampreys are the only vertebrates with an absence of a bile duct system in their livers. This occurs through programmed degeneration of the biliary tract during normal morphogenesis [34]. As the biliary tract regresses, the adult lamprey develops progressive cholestasis and bile pigment accumulation. The spectrum of pathology resembles the human form of the biliary atresia with the accumulation of luminal debris, basement membrane thickening, disorganization of hepatic architecture, extra-hepatic bile duct atresia, and shrinkage or loss of the gall bladder [35]. These animals live for several years after biliary tract regression allowing for studies on compensatory response to cholestasis as well as changes in the evolution of biliary atresia at the molecular level [36].

Bile duct injury has been induced with several agents in an attempt to mimic histological features of biliary atresia. After having identified low levels of L-proline in the serum of patients with biliary atresia, Vacanti and Folkman were able to induce bile duct enlargement with a continuous intraperitoneal infusion of L-proline [37]. 1,4-phenylenediisothiocyanate (PDT), an antihelminthic agent, can be employed to induce bile duct inflammation [38, 39]. The bile duct pathology is related to the timing of exposure to this agent. When gavaged in the postnatal period, PDT causes bile duct enlargement. When gavaged to pregnant rats, PDT causes fibrosis in the bile ducts. However, with a combination of gavage to the pregnant rats and during the postnatal period, the bile ducts exhibit wall thickening with stenosis and atresia. Further study of this temporal relationship may aid in the understanding of bile duct development. Another agent, phorbol myristate acetate (PMA), has been infused directly into the gallbladders of adult rats with a subcutaneous pump [40]. After a 28-day infusion, portal fibrosis and neo-cholangiogenesis were observed. PMA is a nonspecific activator of inflammation and may lead to insights on the role of inflammation in the development of biliary atresia.

Surgical models involving ligation of the fetal bile duct have been described in sheep [41]. Although the distal bile duct can become atretic, similarly to what is found in the human form of biliary atresia, the same does not apply to the impact on the liver, which does not correlate with what is found in the human disease. More recently, it has been shown, also in the ovine model, that occlusion of the fetal bile duct and the consequent hyperarterialization of the liver actually/instead significantly affects hepatic hematopoiesis, leading to a new perspective into the mechanisms that govern hematopoiesis in general, illustrating the potentially far reaching impact of fetal surgical models [42].

3.2.3 Congenital Diaphragmatic Hernia

Multiple animal models of congenital diaphragmatic hernia (CDH) have been described, however only few bear relevance to the human disease.

A model of familial CDH has been described in pigs which were originally bred to produce anorectal malformations, with a prevalence of approximately 10% [43]. Animals show herniated intra-abdominal organs within the thoracic cavity, but not the pulmonary hypoplasia characteristic of CDH. Several genetically manipulated mice models have also demonstrated CDH in combination with other associated malformations. If both murine retinoic acid receptors are deleted, mice have a high incidence of cranial, vertebral, limb, cardiac, foregut and pulmonary malformations, in addition to occasional CDH [44, 45]. Mutations in the homeobox *Hlx* gene result in CDH with large lungs and small livers [46]. Homozygous inactivation of *WT-1* causes CDH and major defects in the urogenital system [47]. Knockout mice homozygous for *Slit3* deficiency exhibit CDH in a ventral midline location with herniation of the liver and gallbladder, along with renal and ureteral agenesis [48].

The first surgical model of CDH was described by De Lorimier using third trimester fetal lambs [49]. Through a maternal hysterotomy and a fetal thoracotomy, a large defect was created in the left dome of the diaphragm. This model resulted in hypoplastic lungs, however with essentially normal pressure-volume curves. Further studies using an inflatable balloon in the fetal chest produced significantly reduced tidal volume and pulmonary compliance compared with control animals [50, 51]. Deflation of the balloon *in utero* improved these pathophysiologic effects and improved newborn survival [50]. In another variation of fetal manipulation, the diaphragmatic defect was created in the second trimester rather than the third, in order to more closely mimic the human disease [52]. In these animals, the lungs were hypoplastic, had abnormal airway branching, and a smaller and more muscularized pulmonary arterial tree when compared with controls [53, 54]. The fetal surgical model of CDH has also been described and further explored in rabbits [55–57]. As these surgical models are created during fetal life, they hold limited significance to the embryogenesis of CDH.

Experimental CDH can also be produced in other animal species through different interventions, other than surgical creation of the defect, including: exposure to diet deficient in either vitamin A [58, 59], zinc [60], or cadmium [61]; administration of either thalidomide [62], anti-rat rabbit serum [63], 2,4-diclorophenil-p-nitrofenilic ether (nitrofen, a herbicide) [64–66], or polibromate biphenils [67, 68]; and genetic manipulations, such as FOG-2, COUP-TFII, and GATA-4 mutations [69–71]. However, with the possible exception to the nitrofen model, there's been no conclusive relationship between these experimental models and clinical/epidemiological data in humans. The nitrofen model has been increasingly accepted as the most relevant to clinical CDH due to the fact that, in that model, the pulmonary hypoplasia precedes the diaphragmatic defect and is independent from the latter. This is in accordance with today's favored notion that the primary defect is not in the diaphragm, but rather in the developing lung buds, with the diaphragmatic defect being actually secondary to a primary pulmonary hypoplasia. Such pulmonary hypoplasia, in turn, could be made worse by the herniated content into the chest.

Laboratory developments in CDH include a peculiar facet which further epitomizes the impact that fetal intervention models can have in our understanding of not only a given disease, but also of germane biological processes. In the sixties, Carmel and colleagues used a healthy leporine model to demonstrate that fetal tracheal occlusion induced lung growth [72]. In the seventies, Alcorn et al. suggested, in a healthy ovine model, that fetal tracheal occlusion and drainage led to hyperplasia and hypoplasia of the lungs, respectively [73]. It was not until the early nineties, however, that Wilson et al. showed, also in sheep, that fetal tracheal occlusion could actually be a means to reverse the pulmonary hypoplasia associated with both CDH and fetal nephrectomy [74–76]. Wilson's sentinel studies on therapeutic fetal tracheal occlusion have triggered one of the most fertile experimental and clinical development sprees of recent memory in our specialty, with ramifications that have crossed the boundaries of our field.

3.2.4 Hirschsprung's Disease

A number of animal species have naturally occurring aganglionic megacolon, including mice, rats and horses [77–79]. In 1966, Lane described two strains of mice with autosomal recessive aganglionosis [79]. The lethal spotting (ls) mice have approximately 2 mm of aganglionosis while piebald lethal (s^l) mice have approximately 10 mm of aganglionosis. Lane and Liu also described megacolon associated with a dominant spotting gene (Dom) in mice, characterized by distal colonic aganglionosis and a long hypoganglionic transition zone [78]. Ikadai and Agematsu described an autosomal recessive total colonic aganglionosis in a strain of rats [77]. These animals have a high mortality rate and are only able to survive for 3–4 weeks after birth, eventually succumbing to severe bowel obstruction and enterocolitis. Histological studies using acetyl cholinesterase whole-mounts in all these rodent

models are virtually identical to the human histopathology [80].

Various genes have been actively disrupted in mice, producing phenotypes similar to human Hirschsprung's Disease (HD). The Ret gene encodes a receptor tyrosine kinase, which has four ligands: glial cell line derived growth factor (GDNF), neurturin (NTN), artemin (ATM) and persephin (PSP) [81]. The complete receptor complex includes the Ret receptor tyrosine kinase and a glycosylphosphatidylinositol-anchored binding component (gfrα1, gfrα2, gfrα3 or gfrα4). This receptor has been suggested to function as an adhesion molecule, which is required for neural crest migration and could also play a role in either differentiation or survival of the neural crest cells which have stopped migrating [82, 83]. Ret (−/−) transgenic mice have a homozygous, targeted mutation of the tyrosine kinase receptor resulting in a loss of its function. These mice exhibit total intestinal aganglionosis and renal agenesis [84]. The Ret gene has been demonstrated to be a major gene causing HD in humans. Mutations of Ret account for 50% of familial and 15–20% of sporadic cases of HD [85–88]. GDNF, one of the Ret receptor ligands, stimulates the proliferation and survival of neural crest derived precursor cells in the embryonic gut [89, 90]. Mice homozygous for null mutation in Ret, GDNF and gfrα1 have almost identical phenotypes characterized by failure of enteric nervous system development distal to the esophagus and absent kidneys [84, 91–95]. Although a causative role for GDNF mutations in some patients with HD has been suggested, the occurrence of such cases is uncommon. It is more likely that the GDNF mutations are involved via its interaction with the Ret receptor [96, 97]. No gfrα1 mutations have been identified in patients with HD [98].

Endothelins are intercellular local messengers that comprise four members to date: ET-1, ET-2, ET-3 and VIP. They transduce a signal via two cell surface transmembrane receptors: ENDR-A and ENDR-B [81]. Both ET-3 and ENDR-B genes have been disrupted and have been identified as the cause for the natural mutants lethal spotting mice and piebald lethal mice, respectively [99, 100]. Moreover, a transgenic mouse ENDR-B knockout has a phenotype identical to the piebald lethal mouse [101]. As the connection between mutations in the Ret receptor and familial HD was established, ET-3 and ENDR-B mutations were also implicated in the disease [99, 100, 102]. However, these mutations have been demonstrated in less than 10% of the cases of HD in humans [103]. Endothelins are initially produced as an inactive proendothelin that has to be activated by a specific enzyme, the endothelin-converting enzyme (ECE). Two ECE genes have been described, ECE-1 and ECE-2 [81]. ECE-1 knockout mice show craniofacial and cardiac abnormalities in addition to colonic aganglionosis [104]. A heterozygous ECE-1 mutation has been identified in a patient with HD who also had craniofacial and cardiac defects [105].

Sox10 is a member of the SRY-related family of transcription factors that is expressed by enteric nervous system precursors before and throughout colonization of the gut mesenchyme [81]. Disruption of the Sox10 gene has been demonstrated to be the cause of the Dom mouse natural mutant [106, 107]. Interestingly, both homozygous and heterozygous animals produce a lethal HD-like phenotype [108]. Mutations in Sox10 have been identified in Waardenburg syndrome associated with HD [109].

Phox2B is a transcription factor that is essential for the development of the neural crest derivates as it regulates the Ret expression in enteric nervous system precursors [110, 111]. Targeted Phox2B gene disruption leads to a complete absence of enteric nervous system in the mice, a phenotype that is very similar to that of the Ret knockout mouse [110]. Garcia-Barcelo et al. reported that Phox2B deficiency might predispose to HD in humans [112].

Pax3 is a member of the paired-box containing family of nuclear transcription factors that is expressed in neural cell precursors giving rise to enteric ganglia and synergizes with Sox10 to activate an enhancer in the Ret gene [113]. In the mouse, Pax3 mutations result in a phenotype characterized by deficient enteric ganglia in the heterozygous state. Homozygous deficient embryos die during mid-gestation

with neural tube defects, cardiac defects and absence of enteric ganglia [113]. So far, no Pax3 mutations have been identified in patients with HD, though.

Most surgical models of HD have involved chick embryos because they are easily accessible and the development of their enteric nervous system has been well studied. In that species, aganglionosis can be caused by surgical ablation of the premigratory neural crest [114]. This model is useful for the investigation of possible treatment strategies. It has been used to recolonize aganglionic bowel with neural crest cells by transplanting tissue obtained from the dorsal neural tube [115–117]. It has also been employed to show that neurons from more proximal regions of bowel are capable of recolonizing distal bowel and forming enteric ganglia [115, 118].

Sato and colleagues described a chemical model of HD [119]. They created segmental aganglionosis by applying benzalkonium chloride topically to the colon and rectum in rats. This model has been reproduced in mice and guinea pigs [120, 121]. It has also been used in the distal esophagus as a model of achalasia [122]. Benzalkonium chloride causes cell damage and death by producing an irreversible depolarization of the cell membrane. Due to the high cell membrane negative charge of neurons, they are more intensely affected then other cells. As a result, benzalkonium chloride induces a selective neuronal ablation in the intestinal wall eliminating almost all myenteric neurons and glia in treated segments [121]. Although the aganglionic bowel does not show hypertrophic nerve bundles and the chemical does not affect the number of submucosal neurons, the treated part does become narrowed and the rectoanal reflex is abolished [119]. Compared to the other models of HD, this technique is inexpensive, easy to perform and the animals can survive longer. It has been used to study functional and structural changes in the bowel resulting from loss of these neural elements. It could also be used to study the chronic changes caused by the aganglionic segment, as well as the long-term effects of different surgical treatments [123–128].

3.2.5 Necrotizing Enterocolitis

To date, no true animal model for necrotizing enterocolitis (NEC) has been described. Nevertheless, as multiple factors have been implicated in the pathogenesis of NEC, several animal models exist that may provide useful platforms for the study of different aspects relevant to the pathophysiology of this disease.

The ischemia/reperfusion model involves direct occlusion of mesenteric vessels or the superior mesenteric artery for varied periods of time followed by reperfusion. It has been performed in different species. In one study in neonatal piglets, the mesenteric vessels were tied off at different points near the distal ileum for 48 h [129]. There was a higher chance of intestinal injury when the occlusion was closer to the ileocecal junction. The degree of injury was greatest in low birth weight piglets as measured by ulceration, vascular engorgement, pneumatosis intestinalis, full-thickness necrosis, and ulceration with perforation. In normal birth weight piglets no injury was observed. This model allows for the investigation of eventual differences in the intestinal response to injury dependent on developmental stages. In mice, the time for the development of ischemic injury following vascular occlusion is substantially less than in low birth weight piglets. For example, occlusion of the superior mesenteric artery for 20 min in adult mice can result in the development of ischemic intestinal lesions in 50% of the animals by 48 h [130].

Studies in human infants with NEC have shown that, within the intestinal lumen, the pH was generally less than 5.0, the protein content less than 5 g/dL, and sufficient carbohydrate and bacteria were available to produce organic acids by fermentation [131]. Based on these data, investigators have created a rabbit model of NEC using a bovine casein formulation acidified with propionic acid [131, 132]. In weanling rabbits, either saline or a solution of 10 mg/mL casein and 50 mg/mL calcium gluconate acidified to a pH of 4.0 was instilled into isolated intestinal loops triggering increased intestinal blood flow, mucosal permeability and histamine release. After 3 h,

the villa were blunted, the lymphatic vessels dilated and edema was observed [133]. After 16 hours, several rabbits had hemorrhagic necrosis and died. Advantages of this model include its simplicity and reproducibility as well as the fact that assorted animals at varied stages of development can be evaluated as to their response.

3.2.6 Short Bowel Syndrome

Perhaps not surprisingly, many models of short bowel syndrome (SBS) have been described. For example, intestinal resection and subsequent gut adaptation have been characterized in the pig [134, 135], dog [136–138], rat [139, 140], and mouse [141]. Warner and colleagues have shown that the murine model can be particularly useful for the study of various genes germane to intestinal adaptation [141]. In this model, a proximal resection is preferred, as adaptive changes are most pronounced in the distal intestine. Large animal models such as the pig are more useful for the development of new surgical bowel lengthening techniques [142–144].

3.2.7 Parenteral Nutrition

Now exceedingly rare due to animal welfare regulations, canine models were instrumental to one of the most relevant achievements not only in pediatric surgery, but in all of medicine and surgery, namely the ability to sustain life exclusively by parenteral nutrition, chiefly through the work of Dudrick and colleagues [145]. In their original study, the aim was to support growth and development in beagle puppies for 10 weeks [146]. Small lipoid pigment deposits and hemosiderin pigment were present in the liver, so dosages of fat and iron were reduced. These results lead to a subsequent study in which 6 beagle puppies were fed entirely by central venous infusion for 72 to 256 days and compared with their littermates [147]. These puppies exceeded their orally fed control littermates in weight gain and matched them in skeletal growth, development, and activity for the study period. The longest-term animals, fed for 235 and 256 days, more than tripled their body weight and developed comparably to their control littermates. These studies first demonstrated that it was both possible and practical to feed animals entirely by vein for prolonged periods of time without excessive risks or compromise of growth and development. Soon thereafter, Dudrick and colleagues administered total parenteral nutrition to six severely malnourished adult patients with chronic, severe gastrointestinal disease for up to 48 days [148]. Positive nitrogen balance was achieved in all of them, along with weight gain, normalized wound healing, and increased activity. All patients were eventually discharged from the hospital. The first neonatal administration occurred in that same year, in an infant with near-total small bowel atresia who underwent a massive intestinal resection [149].

3.2.8 Vacter and Other Models

As previously stated, this was not to be an all-inclusive list, but rather one illustrative of the different development avenues offered by a variety of animal platforms. Other models applicable to the pediatric surgical diseases discussed above, as well as models of interest to other pathological processes, will be discussed in their respective chapters. A special note must be mentioned on the remarkable variety of models of the VACTER (vertebral, anorectal, cardiac, trachea-esophageal, and renal) association, both as far as mechanism of action, as well as variability within the broad spectrum of this "syndrome" [4, 150–159].

3.3 Cell-Based Research

Cell-based therapies remain largely experimental, yet cell-based research has undergone dramatic growth and diversification over the last few decades. Certainly, in light of recent advances in stem cell biology, tissue engineering, gene manipulations, and other so-called regenerative medicine strategies, it is reasonable to speculate that these therapies may become alternatives, if not preferred treatment modalities, for a number

of structural congenital anomalies and other diseases within the realm of pediatric surgery in the not so distant future [160, 161]. The following is a much summarized outline of a few aspects of this burgeoning field that are of particular consequence to our specialty.

Prenatal stem cell and gene therapies have tremendous potential to treat a range of disorders that can be diagnosed or predicted before birth, stemming from the unique environment present during fetal developmental, which can facilitate and enhance cellular engraftment. A notable example is *in utero* hematopoietic stem cell transplantation (IUHSCT). While few disorders have a compelling rationale for IUHSCT based on the prevention of irreversible damage to the fetus before birth, such as for example glycogen storage diseases with neurologic involvement, this methodology can be a powerful means to induce tolerance to transplantation later in life. Flake and colleagues have developed germane work in this area aimed at maximizing chimerism through a variety of strategies so as to achieve complete or near complete replacement of host hematopoiesis by donor cells without toxicity or graft versus host disease in rodent models [162–164]. Consistent results in preclinical large animal models are now being pursued by that group and others [165].

Fetal tissue engineering is another notable development. It constitutes a novel therapeutic concept in perinatal surgery, involving the procurement of fetal cells, which are then used to engineer tissue *in vitro* in parallel to the remainder of gestation, so that an infant, or a fetus, with a prenatally diagnosed birth defect could benefit from having autologous, expanded tissue readily available for surgical implantation in the perinatal period. The fetus is a prime tissue engineering subject, both as a donor and as a host. The many exclusive characteristics of fetal cells, in conjunction with the developmental and long-term impacts of engineered graft implantation into a fetus or a newborn, add new dimensions to tissue engineering generally. Also, the fact that certain congenital anomalies present as perinatal surgical emergencies further justifies the fetal tissue engineering principle. Our group and others have been developing this notion in a variety of animal models of structural congenital anomalies, typically employing the amniotic fluid as a preferred source of fetal cells [166–180]. Preclinical studies have been reported and the first clinical trials are expected for the near future [181–183]. Another facet of fetal cell-based treatments of structural anomalies being developed experimentally is the use of fetal neural stem cells for the repair of spinal cord damage in the setting of neural tube defects, such as spina bifida [184]. Additionally, select fetal cells have also been proven valuable experimentally in studies on wound healing modulation [185].

Tissue engineering techniques have already been used to repair congenital anomalies postnatally in children. Shin'oka and colleagues have accumulated considerable clinical experience with the use of engineered conduits as vascular replacements in low-pressure systems, in children with varying forms of complex congenital cardiovascular anomalies [186–190]. Further clinical experience with tissue engineering in pediatric surgery beyond the more prevalent anecdotal reports is expected in the coming years.

More recently, transamniotic stem cell therapy (TRASCET) has emerged experimentally as a novel therapeutic strategy for the treatment of different birth defects. It is based on the principle of harnessing/enhancing the normal biological role of mesenchymal stem cells that are naturally occurring in the amniotic fluid for therapeutic benefit. Specifically, we have recently shown that amniotic fluid-derived mesenchymal stem cells (afMSCs) play a central role in fetal wound healing, widely known to be enhanced when compared with postnatal repair of tissue damage [185]. This germane finding was not only the first demonstration of a biological role for any amniotic cell, it has also provided validation for the use of afMSCs in regenerative strategies, in that these cells already play a regenerative role in nature. More recently, we have also shown, in different animal models, that the simple intra-amniotic delivery of afMSCs in large numbers can either elicit the repair, or significantly mitigate the effects associated with major congenital anomalies, putatively by boosting the activity

that these cells normally have. For example, concentrated amounts of these cells injected into the amniotic cavity can induce partial or complete coverage of experimental spina bifida by promoting the local formation of a host-derived primitive skin, thus protecting the spinal cord from damage [191, 192]. Placenta-derived MSCs also seem to be a suitable option for TRASCET, at least in experimental spina bifida [193]. In another example, TRASCET has been shown to significantly alleviate the bowel damage associated with gastroschisis [194]. Many other applications of this practical therapeutic concept, involving a variety of congenital anomalies, are currently being investigated.

3.4 Clinical Research

Clinical research has evolved appreciably, particularly over the last two decades. It has essentially become a science deserving of a whole book, rather than a segment of a book chapter. The several aspects that make up clinical research need careful planning and execution if a study is to be any relevant. More specifically, conceiving the research question(s); establishing the appropriate study/trial format; defining randomization criteria when suitable; choosing and recruiting the research subjects; estimating sample size and power; assessing control/independent variables and/or causal interference; designing questionnaires and interviews; organizing and managing databases; analyzing data; implementing quality control; and addressing ethical issues are just some of the components that need to be tackled before one can embark on a meaningful project. By the same token, as the clinical research endeavor becomes more refined, it expectedly subdivides, perhaps more notably between clinical trials and outcomes research.

As critical as it is to any medical/surgical field, the overall adequacy of clinical research design and reporting in our specialty has been rather inconsistent over time [195]. Fortunately, however, pediatric surgeons have grown increasingly more discerning of late, progressively driving our scientific journals and professional societies to implement enhanced and more standardized peer-review guidelines which ultimately should be of great benefit to the field as a whole [195–199].

3.5 Final Considerations

The history of our young specialty is already rich in original translational initiatives which have shaped clinical practice both within and across the boundaries of our field. Among the many of these, perhaps one should stand out as an inspiration to all of us. A pediatric surgeon, Dr. M. Judah Folkman, was the first to propose and coin the term "antiangiogenesis" as a potential therapeutic approach to cancer and other conditions in his landmark paper of 1971 [200]. With this seminal insight, he established a new perspective on cancer biology by expanding the focus beyond the tumor cells to their microenvironment. The concept that proliferating endothelial cells may be better therapeutic targets than the neoplastic cells themselves represented a momentous shift of focus and triggered an enormous research enterprise. Folkman's direct and rational approach to angiogenesis redefined cancer biology, as well as multiple other processes in health, embryonic development, and other diseases [201]. It has been predicted that angiogenesis-related therapies can eventually benefit half a billion people worldwide [202].

As Dr. Folkman used to say, "science goes where you imagine it". Let us hope that more and more of our colleagues can be drawn by that inspirational vision and manage to incorporate either of the many forms of pediatric surgical research into their daily activities and ambitions.

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