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Adaptation: Paradigm for the Gut and an Academic Career

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Abstract

Adaptation is an important compensatory response to environmental cues resulting in enhanced survival. In the gut, the abrupt loss of intestinal length is characterized by increased rates of enterocyte proliferation and apoptosis and culminates in adaptive villus and crypt growth. In the development of an academic pediatric surgical career, adaptation is also an important compensatory response to survive the ever changing research, clinical, and economic environment. The ability to adapt in both situations is critical for patients and a legacy of pediatric surgical contributions to advance our knowledge of multiple conditions and diseases.

Keywords

short gut syndrome; intestinal resection; surgical research; pediatric surgery; intestinal adaptation

A biological definition of adaptation is any alteration in the structure or function of an organism resulting from natural selection. Thus, the organism becomes better fitted to survive and multiply in its environment in response to environmental cues. Since most of my academic career has been focused on understanding adaptation responses of the remnant intestine to massive intestinal loss, I have also realized a similar paradigm with regard to the importance of adaptation for the development of a successful academic career. In the following dialogue, I will first present data that our laboratory has generated over the past several years with regard to resection-induced adaptation responses. Next, I will present parallel observations with regard to my own career development and how this may be applied to others pursuing investigative careers in pediatric surgery.

My overall interest in adaptation began as a result of caring for patients with short gut syndrome during my pediatric surgical fellowship. I was impressed how the loss of a significant length of this digestive organ profoundly impacted the quality of life and long-term survival of these patients. While there are recent indications that survival is improving in children who have suffered massive intestinal loss (1), the overall survival probability is directly related to bowel length and remains poor beyond five years after massive intestinal resection. In adults who have less than 50 cm of small intestine length, long term survival is in the range of 40% (2). Intestinal lengthening procedures include the Bianchi technique (3) and serial transverse enteroplasty (4) have enabled a subset of patients to wean completely

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from parenteral nutrition. However, postoperative intestinal length is still a major limiting factor influencing survival. Short-term outcomes for intestinal transplantation have improved in recent years, but long term survival still ranges between 40-60% (5,6,7).

Intestinal adaptation

Intestinal adaptation is an important response to massive small bowel resection (SBR) and represents a mitogenic signal to the intestine culminating in an expanded mucosal digestive and absorptive surface area. The expression of several immediate-early genes within the remnant bowel has been recorded to be elevated within hours of intestinal resection (8,9). Similarly, our laboratory has demonstrated physical changes in the remnant bowel as soon as 24 hours after SBR in our murine model, but prior to the initiation of enteral feeding (10). Adaptation is structurally characterized by taller villi and deeper crypts (**Figure 1**), as well as enhanced rates of enterocyte proliferation and apoptosis. While these features are a renowned characteristic of adaptation in animal models of massive SBR, similar structural alterations have not consistently been described in humans. To address this, we studied the intestine in a uniform population of infants with neonatal necrotizing enterocolitis who required intestinal resection (11). Comparing villus height and crypt depth at the normal margin of tissue at the time of resection with the same measurements at the time of ostomy takedown revealed significant increases in both parameters. We therefore feel that these findings validate animal models for studying mechanisms of adaptation.

The mechanisms and mediators of intestinal adaptation are multi-factorial and include intraluminal nutrients, gastrointestinal secretions, as well as hormones (12,13). The most compelling evidence for the role of hormones was provided by a surgical model of vascular parabiosis in which two rats share a common circulation (14). In that report, intestinal resection in one animal resulted in adaptive changes in the intestine of the other unoperated animal. Multiple humoral factors that have been suggested to play a role in intestinal adaptation and include epidermal growth factor (EGF), glucagon-like peptide-2, insulin-like growth factor, leptin, thyroxine, growth hormone, and corticosteroids, to name but a few (15). Many of these factors have either been found to be elevated in the serum of patients who have undergone SBR, or infusion of these agents following SBR has resulted in enhanced parameters of adaptation.

Epidermal growth factor and adaptation

We began our studies of intestinal adaptation using EGF. This was truly an adaptive response to our environment, since we had a fair amount of EGF available in our laboratory from another investigator working on a different project. One industrious pediatric surgical research fellow in my laboratory infused EGF to rats after SBR and demonstrated significant increases in weight gain as well as other parameters of adaptation (16). This seminal observation took great advantage of an available environmental resource (left over EGF) and set the stage for many years of further research.

In order to further our understanding of adaptation and the role for EGF, we characterized a murine model for intestinal resection (10). The advantage of the mouse is that it can be genetically manipulated to more directly test the contribution of specific genes within select cell populations and their roles in specific adaptation responses. Through several experimental paradigms, we found that stimulation of the EGF receptor (via exogenous EGF (17)), in EGF transgenic mice (18) or administration of another EGF receptor ligand (transforming growth factor alpha (19)) resulted in enhanced adaptation responses. Alternatively, inhibiting EGF receptor signaling by removing the submandibular glands – a major source of endogenous EGF in the mouse (20), performing SBR procedures in

waved-2 mice with faulty EGF receptor activity (21), or administration of a pharmacologic EGF receptor inhibitor (22) all resulted in attenuated adaptation responses.

Having established the relevance of this receptor, we began to further elucidate specific mechanisms for how EGF receptor signaling regulates adaptation. This required the refinement of techniques for recovery of cells from the intestine. Prior studies employed whole bowel homogenates or mucosal scrapings for purposes of detecting alterations in the expression and/or activities of specific genes and proteins in the adapting bowel. Unfortunately, since there are multiple different cell types within the intestinal wall, the potential to overlook important changes within specific cellular regions is great. We therefore learned the technique of laser capture microdissection (LCM) microscopy in order to detect changes in mRNA expression within the proliferative zone of enterocytes in the crypt versus the post-mitotic, differentiated cells of the villus (23). We have subsequently advanced a technique for isolation of intact crypt and villus units which provides a greater cellular yield for protein analysis (24) (**Figure 2**).

The balance of enterocyte proliferation and apoptosis

The intestinal mucosa is a very dynamic organ, containing some of the most rapidly proliferating cells in the body. The relationship between the rate of cell production and cell death must be precise, since any imbalance may result in either intestinal mucosal atrophy or neoplasia. Initial studies therefore focused on EGF receptor regulation of enterocyte proliferation. In vitro, we found that expression of the cell cycle inhibitor p21^{waf1/cip1} (p21) was increased and paradoxically required for EGF-directed enterocyte proliferation (25). We mapped a critical region of the p21 promoter that was activated by EGF receptor stimulation. This promoter activity required activated extracellular signal-regulated kinase (ERK) 1/2 and contained a putative binding site for the transcription factor Sp1. The requirement for this cell cycle regulatory protein was verified in earlier experiments in which p21-null mice demonstrated no induction of enterocyte proliferation after the stimulus of SBR (26).

In seeking potential mechanisms for how p21 regulates adaptation, we initially expanded upon a related observation that p21 affects stem cell populations within bone marrow (27). We therefore sought to determine the effect of p21 deficiency on intestinal stem cells (28). In these experiments, we were unable to demonstrate differences in the expression of candidate stem cell markers or numbers of crypt-base columnar cells in p21-nulls versus control mice. However, we did identify increased expression of another cell cycle inhibitor Retinoblastoma protein (Rb) within the crypt cells of the p21-deficient mice (29). The significance of elevated Rb expression was established by genetically inactivating a single Rb allele in the p21-null animals, which restored enterocyte proliferation and adaptation responses. Further, we demonstrated that rates of enterocyte proliferation and villus growth were magnified when Rb expression was completely disrupted within the intestinal epithelium (24). Ongoing experiments are now focused upon illuminating this previously unrecognized role for Rb as a critical player in the molecular mechanism of resection-induced enterocyte proliferation and adaptation.

In parallel with studies delineating mechanisms of enterocyte proliferation, we identified increased rates of enterocyte apoptosis following SBR (30). We were initially perplexed at this observation since the primary role for adaptation is to grow mucosa, not promote increased cell death. However, since rates of enterocyte production must be perfectly matched by rates of enterocyte loss, our findings made biological sense. Since apoptosis is programmed cell death and is genetically regulated, we initially tested the hypothesis that expression of the proapoptotic Bcl-2 family member Bax would be affected by SBR. Indeed,

we found that Bax expression was elevated after SBR, coinciding with reduced expression of an anti-apoptotic Bcl-2 family member Bcl-w (31). The role for EGF receptor regulation of Bax expression was verified in waved-2 mice with impaired EGF receptor signaling capacity in which we demonstrated exaggerated rates of apoptosis after SBR (32,33). Alternatively, we found that EGF administration attenuated Bax expression and resection-induced apoptosis (32,34). Using LCM microscopy, we were able to target the proliferative crypt compartment as the site for the greatest changes in Bax and Bcl-w expression (35). The significance of Bax expression and resection-induced apoptosis was established in Bax-null mice in which the apoptosis response is prevented (36).

We next pursued understanding the mechanisms for EGF receptor regulation of Bax. In cultured intestinal epithelial cells, we found that inhibiting EGF receptor signaling resulted in increased apoptosis and enhanced Bax activity (37). In these experiments, we discovered that EGF receptor inhibition resulted in activation of p38- α mitogen-activated protein kinase (MAPK), which was then necessary for increased Bax activity and apoptosis. Extending these observations into the whole animal, we generated intestinal epithelial-specific p38- α knockout mice and then performed intestinal resections (38). Consistent with our *in vitro* studies, Bax activity and apoptosis were both prevented after SBR in p38-null mice.

The ultimate utility of focusing on both the rates of proliferation and apoptosis is that future growth factor and/or pharmacologic therapy targeted to stimulate proliferation while at the same time inhibit apoptosis may result in an even greater expanded mucosal surface area than either intervention alone. The benefits of this dual therapeutic approach was suggested by co- administration of EGF (to inhibit apoptosis and stimulate proliferation) and a pharmacologic apoptosis inhibitor after SBR resulting in greater mucosal growth than either treatment (39). Explicit understanding of these mechanisms will be necessary for optimization of this novel therapy.

Angiogenesis

We have previously identified a significant induction of capillary growth within adapting intestinal villi (40). It is presently unclear whether this angiogenic response occurs as a result of stimulated enterocyte production or whether this angiogenic response is a primary signal to induce enterocyte proliferation. In initial experiments, we established an important collaboration with our colleagues in biomedical engineering to adapt their photoacoustic microscopy technology (41) to determine actual blood flow within capillaries of the intestinal wall following SBR (Rowland et al, in press). Indeed, we found that blood flow and arterial oxygen saturation were reduced immediately following SBR and intestinal oxygen extraction was elevated. It is therefore possible that the immediate response to SBR results in a hypoxic milieu which may initiate a series of hypoxia-regulated genes capable of signaling for enterocyte proliferation.

Intestinal smooth muscle

In addition to investigating the adaptive role of angiogenesis to small bowel resection, we have investigated the contribution of intestinal smooth muscle. Enteric smooth muscle appears to adapt much like the mucosa. There is a modest intestinal lengthening response as well as an increase in intestinal caliber (42). We adapted to our environment at the University of Cincinnati and collaborated with researchers in the adult Endocrinology Division who had a mouse line in which insulin-like growth factor-1 (IGF-1; another growth factor similar to EGF) is overexpressed within smooth muscle cells. When we performed SBR in these mice, we found that adaptive intestinal lengthening was almost doubled compared to the nontransgenic littermates (43). This observation challenges years of focus

on enterocytes as a key driver of adaptation. Perhaps the enterocytes lining the mucosa may actually be proliferating in response to underlying mesenchymal growth as a secondarily response. The focus on intestinal smooth muscle is therefore a very important consideration for future studies.

Metabolic effects of adaptation

The changes that occur in the whole body during adaption are not well characterized. Using indirect calorimetry as well as body composition analysis, we recently identified significant changes in lean body mass as well as fat stores in mice after SBR (44). Not surprisingly, we noted that lean body mass falls after SBR, but never really catches up to the sham operated group. On the other hand, while fat stores are initially depleted after SBR, fat stores are restored back to normal levels over time. This response is unusual and not obviously beneficial for the host since biology would predict that restoration of lean body mass would have priority over fat.

Adaptation for Success in Career Development

With an ever-changing landscape for pediatric surgery training as well as the clinical field itself, the significance of research seems to be increasingly marginalized. There are several points that are relevant with regard to pediatric surgeons doing research. First, I feel deeply that it is our responsibility to do our best for our patients. As such, the exercise of constantly questioning is fundamentally important. The research of today will be the practice of tomorrow and improving outcomes will require vigorous hypothesis-driven investigation. Refining surgical techniques are important but it is my belief that for many conditions in pediatric surgery, the actual technique of *how* we do a procedure will have less of an impact than understanding *why*. Several enigmatic conditions in pediatric surgery demand both basic and clinical investigation. These include congenital diaphragmatic hernia, necrotizing enterocolitis, and biliary atresia. I would submit that whether we repair the diaphragm, resect the bowel, or excise the extra hepatic biliary tree via open, laparoscopic, or robotic techniques, the fundamental pathogenesis is going to be much more important for us to understand. Scientific investigation is therefore crucial to advance our understanding of the many conditions that we treat on a daily basis.

The concern about spending additional time training in research is real. Seven clinical years of training after medical school to become a board-eligible pediatric surgeon is quite a long time. On the other hand, our field has appropriately resisted recommendations to reduce the number of clinical training years with the rationale that the spectrum of our clinical field spans many subspecialties. Unfortunately, the current reductionist environment forces us to challenge the paradigm of surgical residents spending additional years learning research. I would argue that just because our specialty requires a longer period of clinical training does not mean we should ignore the value of a research exposure during residency. Our field absolutely needs it. Many spend time in a laboratory for the sole purpose of securing a pediatric surgical fellowship. Indeed, several studies spanning two decades have verified that the number of publications and research experience remain strong predictors of success in the pediatric surgical match (45,46,47). I would argue that this is a very important component of surgical resident training. This often may be the first and perhaps only exposure to research for surgical residents. This was certainly the case with me. Without this initial exposure, I doubt that I would be doing what I am doing.

The early exposure to research also provides essential training in experimental design, understanding scientific techniques, and the ability to gain a deep understanding of a focused clinical problem. In addition, the research time provides the opportunity to learn to read critically as well as develop presentation and organization skills. I would argue that

even if a trainee never does research again, these learning points are widely applicable. Another significant factor for residents doing research is that they really do contribute to the overall research program. Residents are remarkably productive and very hard working. They are often coming up with new ideas or directions to the laboratory mentor. Their strong work ethic makes them a highly valued team player to move the mission of the laboratory forward. Further, it is during this time that the residents are able to demonstrate their academic potential. Finally, the research time often provides a break from busy clinical responsibilities in which residents have more control over their own schedules.

The concept that successful research is becoming less frequent is a harsh reality that is realized for all researchers – regardless of medical subspecialty. It is also true for non-MD researchers. These are simply tough economic times. On a more positive note, I have recently identified 40 NIH-funded pediatric surgeons out of our current 455 active APSA members. This means that 8.7% of active pediatric surgeons are presently receiving NIH support for their research. This does not include other important sources of funding for research such as the Department of Defense, March of Dimes, etc. By comparison, there are a total of 994 NIH awards to Departments of Surgery throughout the United States (<http://projectreporter.nih.gov/reporter.cfm>). As a percentage of American College of Surgeons membership (roughly 70,000), that represents only 1.4%. While I fully recognize the limitations of this comparison, the point is that most pediatric surgeons have had research experience during their training. This has culminated in a significantly greater fraction of surgeons in our specialty with subsequent active NIH support. Something must be working.

“If you are not failing every now and again it is a sign that you are not doing anything very innovative”

--- Woody Allen

Research is difficult, not always fun, and frequently unsuccessful. It does require adaptation. One of the biggest adaptations is to be able to step back and appreciate the fact that your original hypothesis may actually be incorrect. In these circumstances, rather than throwing in the towel, it is critical to question further. Negative data is just as important as positive data if done correctly. Stated another way, the answer “no” is just as important as “yes” – providing the question is good one.

“Nothing in this world takes the place of persistence. Talent will not, nothing is more common than unsuccessful people with talent. Genius will not, unrewarded genius is almost a proverb. Education will not, the world is full of educated derelicts. Persistence and determination alone are omnipotent.”

--- Calvin Coolidge

30th President of the United States.

My own research program has advanced despite a long trail of rejections from multiple societies, journals, and grant agencies for what I have considered to be our most seminal work. In retrospect, because I have desperately sought to improve as a result of this negative feedback, I feel that our research is far better. The above quotation sits on the door to my office and epitomizes the most important aspect of success in research – keep working at it!

Mentorship is critical. It is important to appreciate that there are usually several different mentors. It does not have to be a single pediatric surgeon or a basic scientist. Mentorship comes in all forms and at many levels along the way. For young pediatric surgeons early in their career, it is important to seek mentors who have experience and a track record of success..

The environment is also important. An ideal environment is a strong academic medical center where there is a deep culture of collaboration and the availability of multiple core facilities. It is also imperative to work within a group that respects and appreciates the research effort. There has to be an element of accountability. Faculty with protected time for research must use that time for research. It is easier to spend longer times with clinical care. Certainly, the rewards are much more immediate and there is a greater comfort level when dealing with patient issues as opposed to specific research questions. On the other hand, using clinical time as a surrogate for investigative effort will lessen chances for research success.

With regard to the project, it is important to avoid intellectual paralysis. Many young researchers are afraid to actually start doing experiments until they feel as though they have read everything possible. This turns into a vicious cycle of the more that is read, the more the investigator feels the need to read. Ultimately there is burn-out without any sort of forward movement. While a comprehensive understanding of the literature is essential, sometimes it is better to just take a step and do an experiment. Despite the fact that EGF is a central theme for many of our experiments, I wholly recognize that EGF is not the Holy Grail for adaptation. Despite this, it has been a great vehicle to navigate through the labyrinth of understanding adaptation responses and contributing new knowledge.

Collaboration is central with basic scientists as well as individuals with clinical expertise in other disciplines. The research projects will be better. They will also be viewed more favorably by granting organizations. The ability to collaborate and build bridges will lead to better questions, state of the art techniques, and better interpretation of the data that is generated. This is optimized by working collaboratively across multiple disciplines. I have had the privilege of working closely with Christopher Erwin, Ph.D. and Jun Guo, Ph.D. in our laboratory for many years. They have been the glue for our research program and have successfully adapted to a major institutional move as well as countless new techniques and challenges.

Finally, I think it is important to understand that life balance is crucial for academic success. Nothing can take the place of a supportive and understanding partner/spouse/family. A life outside of the hospital/laboratory is essential. Happiness at home will provide a much greater likelihood of success at work and vice versa.

While our own laboratory contributions may not always have the most major impact, stepwise and persistent contributions may someday provide essential elements for a more thorough understanding of intestinal adaptation. Our research program has been built around persistence and ability to adapt to changing techniques, environments, mentors, and data which consistently challenge our hypotheses and ideas. It is these features that will be enable improvement in the care of children with short gut syndrome. When applied to other conditions and diseases, our adaptive potential will undoubtedly enable us to continue to make progress.

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Biography

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Figure 1. Structural features of intestinal adaptation

When compared with the ileum removed from a mouse after a sham operation (A; bowel transection with reanastomosis alone), removal of 50% of the proximal jejunum results in significant villus growth as well as crypt deepening at the 3 day postoperative time point (B).

Figure 2. Isolation of crypt and villus units from mouse ileum

We have refined a technique for isolation of pure populations of crypt (A) and villus (B) enterocytes (24). This technique allows for more precise understanding of genetic alterations within specific cell populations within the bowel in response to intestinal resection.