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Jay and Margie Grosfeld Lecture

A pain in the NEC: Research challenges and opportunities

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ABSTRACT

This lecture will describe how the properties of heparin-binding EFG-like growth factor (HB-EGF) can be utilized to protect the intestines from necrotizing enterocolitis (NEC), to augment the effects of stem cells in the treatment of NEC, and to improve the production of tissue-engineered intestine. It will also explore the role of the enteric nervous system in the development of NEC.

Dr. Krummell, Dr. and Mrs. Grosfeld, members, and guests — when Dr. Krummel called me months ago and invited me to deliver the 2014 Jay and Margie Grosfeld Lecture I was stunned. I immediately thought of the previous deliverers of this address, each of whom gave powerful lectures, particularly recalling the 2013 lecture delivered by Dr. Jessica Kandel. As I sat in the audience listening to that lecture I vividly recall thinking that the 2014 lecturer would have impossibly big footsteps to follow. Gratefully, it never entered my mind at the time that it would be me, but here I am. I sincerely thank Dr. Krummel for the opportunity to share with you today a little about my research journey. Furthermore, I am deeply indebted to Jay and Margie Grosfeld for the honor of delivering this lecture in their names, and we are all grateful for everything Dr. Grosfeld has done over the years to support APSA and the ability of young surgeons to perform surgical research. He has worked tirelessly eliciting donations to the APSA foundation, which go directly to support research enrichment scholarships. The APSA foundation scholarship was one of the first research awards I ever received, and was certainly instrumental in my ability to subsequently obtain additional external funding for my research. One thing I can guarantee you is that if Dr. Grosfeld has not already approached you to donate he will do so soon, so be prepared to give! As you can see from this list of award winners, the return on investment is quite good.

The title of my talk today is "A pain in the NEC: Research challenges and opportunities." Please don't interpret my use of the term "A pain in the NEC" as being flippant in any way — rather it reflects the pain that we all have to deal with when we operate on one of these patients and have to tell the parents that we don't think that their baby will survive. The first case of NEC that I ever operated on was during the third month of my first year as a pediatric surgery fellow. Baby boy Freeman, a 900 gram premie, survived the operation and lived, and I was elated. Today, so many years later, I hope to never have to operate on one of these babies again. The second half of the title – research challenges and opportunities – reflects the ups and downs that we all face when doing research.

As I prepared for this lecture I reflected on what it was that actually instilled in me a passionate love for research. Could it be that the love of research is something one is born with? If you do a Google search of "Besner" in order to find the very first paper written under this name, you will not find any of my prior studies of growth factors, intestinal injury, or anything even written by me. You will find this paper, entitled the law and low level radiation, which was presented at the congress of the international radiation protection association in Washington, DC in 1973. The purpose of the paper was to examine a number of latent radiation injury cases with emphasis on the kinds of radiation records offered in evidence, the nature of the expert testimony, and the conclusions of the court. You will see that the very first case that was successfully won in court that linked repetitive exposures to low-dose radiation at the workplace with cancer was a New York case of an employee who was a theoretical physicist who died from acute myeloblastic leukemia, referred to as reference number 30 [1]. Examination of the reference list shows that this case was Besner *vs.* Walter Kidde Laboratories. Quoting from the paper, "the record discloses that decedent was exposed to radiation for a substantial part of two periods and also at other times in various amounts. The testimony of the medical experts is emphatic

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Fig. 1. Aaron Besner, PhD (1923–1958). Besner *vs.* Walter Kidde Laboratories (1963) confirmed that repeated exposure to low-dose radiation in the workplace was linked to the development of cancer.

that there is really no threshold or safe dosage of radiation because at the present stage of scientific knowledge it cannot be ascertained exactly what effects radiation has on the human body. The award is supported by substantial evidence ….especially so in view of decedent's good health prior to his employment."

None of you in the audience will recognize this man (Fig. 1). This is Dr. Aaron Besner born in 1923. He was a nuclear physicist who worked for Walter Kidde Nuclear Laboratories in New York City. His project was to design and build an atomic-powered locomotive. These were in the days before the dangers of low-dose radiation were understood or able to be monitored. After radiation exposure in the research lab, he was diagnosed with acute myeloblastic leukemia in 1957 and died in 1958 at the age of 35. As you probably figured out, Aaron Besner was my father, who I never know since he died when I was a baby. Although I never knew my father growing up, could he have left me with an innate love for research?

I was raised by my mother Dorothy Besner, a single parent in the heart of New York City. I remember her telling me that science was "in my genes." When I was in high school I told her that I wanted to be a scientist. Having lost her husband to the field of science, I can only imagine what she must have thought. She was very supportive of my career decision, but told me that she thought I should consider doing research after first becoming a doctor, so that I would understand what problems patients have that really required research. Although I didn't understand it at the time, my mother, who had absolutely no medical knowledge, was describing a physician scientist. Despite never wanting to listen to anything my mother told me to do, I took this piece of advice, and I have never regretted it.

I did my general surgery residency at the Brigham & Women's Hospital in Boston. Every year, Dr. Judah Folkman crossed over the bridge from Boston Children's Hospital to the Brigham to give a talk to the surgery residents. As soon as I heard him speak I decided that no matter how long it took, I wanted to do research in his laboratory. As you might imagine, many other people had the same idea, so it took me until after my 5th year of residency to get a research fellowship in his lab, but it was well worth the wait. At that time in the Folkman Lab it was well known that angiogenesis, or the growth of new blood vessels, was controlled by potent angiogenic growth factors such as basic fibroblast growth factor (bFGF). However, bFGF was known to be cell associated and not secreted, so it was difficult to understand how it could go about exerting its potent effects if it could never even get out of cells. I was given a project to find a cell type that could naturally secrete basic FGF. Having done some research on macrophages as a college student, I immediately thought of looking at macrophages, since these cells are highly secretory in nature. Early every morning I would arrive at the Dana Farber Cancer Institute when they were spinning off the buffy coats from units of blood that had been donated, I brought these human white blood cells back to the lab and from them I purified macrophages such as those shown here. After a year of working on these cells, I was convinced I had done it — I had identified a heparin-binding growth factor that was naturally secreted from macrophages that I thought was basic FGF. However further studies showed that it had some properties that were different from FGF. During my second year in the lab it was sadly apparent that I had not identified a secreted form of FGF at all. However, further analysis showed that I had serendipitously discovered a totally new growth factor which came to be known as heparin-binding EGF-like growth factor, or HB-EGF.

This was the first report of HB-EGF that appeared in the literature in 1990 [2]. HB-EGF is produced as a 208 amino acid precursor molecule that undergoes extracellular proteolytic cleavage to yield the mature secreted form of the growth factor. HB-EGF contains an EGF-like domain and an N-terminal hydrophilic extension in which its ability to bind to heparin resides. Here we can see the structures of the molecule that were later elucidated. Since our initial description of HB-EGF in 1991, the number of HB-EGF publications world-wide has increased annually, and includes reports showing that HB-EGF protects the brain from stroke, the heart from myocardial infarction, and the kidneys, bladder and liver from various forms of injury. Over the past two decades, our laboratory has focused on studies showing that HB-EGF can protect the intestines from various forms of injury including necrotizing enterocolitis (NEC) [3].

As pediatric surgeons, we are frequently confronted with the often devastating disease of necrotizing enterocolitis. This illustration depicts the typical appearance of a baby with NEC. These patients are usually born prematurely, and you can tell from the number of lines, tubes and drains that are in place that the baby is quite ill. Here we can see several loops of bowel that are severely injured, and if you examine the intestine closely you can see bubbles of air trapped in the bowel wall, produced by gas-forming bacteria, known as pneumatosis intestinalis. Despite the most advanced methods of caring for these patients including surgical removal of the diseased intestines, and despite over six decades of research, the mortality of NEC remains unchanged and is as high as 50%. Sadly, NEC is still a disease for which there is currently no known cure.

In the past two decades, our laboratory has used multiple different animal models to examine the effects of HB-EGF on the intestines, including animal models of necrotizing enterocolitis, intestinal ischemia/reperfusion injury, hemorrhagic shock and resuscitation, cecal ligation and perforation, radiation therapy-induced intestinal injury, and intestinal anastomotic wound healing, and we have shown that HB-EGF protects the intestines from injury in the first 5 models and promotes healing in the 6th model [4–9]. This is what our rat pup model of NEC looks like. Rat pups are delivered by C-section prior to term, and starting shortly after birth are exposed to hypoxia in a hypoxic chamber followed by hypothermia, and with the addition of hypertonic feeds and lipopolysaccharide, we see an appearance of the intestine that is very reminiscent of the clinical disease that we see in the operating room. When HB-EGF is administered to the pups, we add it directly to