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Views of Translational Research from a Somewhat Translational Scientist

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Abstract

This review arose from a talk entitled “Identifying Targets” and given by the author at EB2011 at the invitation of the American Federation for Medical Research (AFMR). The presentation was part of the AFMR workshop entitled “Keys for Translation: Science and Strategy” and focused on identifying clinically relevant targets as a result of observations made during basic scientific studies. The review emphasizes that targets do not have to be the aim that drives basic discovery, but communication between the basic scientist and clinical investigators may aid recognition of such targets and their translation to clinical applications. Using one line of investigator-initiated research from his own laboratory as an example, the author emphasizes that basic discovery must be hypothesis driven and allowed to follow its logical sequence. Finding treatments, while always an aim of biomedical research, may arise as a result of basic studies that were not originally aimed at a target of translational research.

Introduction

My perspective of the opportunity to get involved in translational research comes from one who may not fit the definition of a “translational researcher”, but it may be that no one quite knows what someone else means by that title anyhow. Thus, when I was invited to share my insights at the AFMR Translational Medical Research Workshop at EB2011, I chose to describe how my own research got to its current state in hopes that this reminiscence would encourage others to consider how they might identify targets for translational research in their own work.

At the end of my name there is an MD rather than a PhD. I was trained in clinical science before going into the basic science laboratory, but I am nonetheless a basic scientist. From that frame of reference I’d like to consider what we do, why we do it, and how we may be able to integrate efforts towards our ultimate goals for basic research.

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Discovery

One clear goal that influences everything we do is discovery. When each of us starts in medical or graduate school or in the basic science laboratory, we start doing something that likely will drive our interest for the rest of our lives. I suspect that for many of us that something is a quest for discovery, for something new that we may be able to identify or create.

However, discovery doesn't do very much good if you don't do something with it. So what can we do with discovery? It might be that the discovery is simply something that allows us to satisfy our own intellectual curiosity. A mentor during my Neurology training used to talk about clinical diagnoses, which are themselves rather like hypotheses, in terms of a rank order. His said that we as clinicians want to make a correct diagnosis. Making the diagnosis most likely to be the reason for the person's illness is the top rank. However, a second order is consideration of diagnoses that, if missed, have a high likelihood, of causing harm to the patient or fatality. The third rank, and this one I loved, includes diagnoses that keep us interested in our field so that we maintain intellectual curiosity. The thinking is similar in the laboratory, where we focus on new things that may vitalize our curiosity and interest in our field of science while hopefully moving that scientific field forward with both likely and unexpected outcomes. Maintaining a fertile intellectual field provides a place for discovery to grow even when the discovery may be unrelated to the original goal of our research. James Austin recognized this in his nice work "Chase, Chance and Creativity"¹ in which he emphasizes that the chase for discovery can be met by futility if the chance discovery does not come to a mind prepared for creativity. Isaac Asimov also emphasized preparedness for uniqueness when he said, "The most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!' (I found it!) but 'That's funny'--".

As a clinician trained in the way diseases may present and in the classic understanding of Physiology I have had a number of Asimov moments. Those "funny" differences from what was expected have not only led me down some of the most interesting trails of science but also have opened opportunities to consider translating discovery into clinically important possibilities.

Discovery really is the beginning of a never ending road on which one discovery simply provides a sign to the next. Many times we wish the speed limit were a bit faster as we strive to take our discoveries from the bench to the bedside either ourselves or through the work of others in the future. Keep in mind that the term for what we do is "REsearch", and I emphasize "RE"-search because we must always search discoveries of those before us in order to apply what has been learned to the questions that we're asking today. Like a fine wine, discovery only reaches its full measure when aged by time and conditions and then appreciated by the right person(s).

Much as we might wish that today's discovery will lead directly to a cure, there often must be further knowledge before translation can occur. The novel *The Century of the Surgeon*² emphasizes that point when it describes a fictional surgeon's life woven through the history of surgery as if he had lived through each of those days. In the story the surgeon's wife

became ill. Despite his considerable skills and despite how much medicine and surgery had advanced he was unable to help his wife who died. The novelist realized that as much as we may want to know how to cure a particular disease, it takes time for us to arrive at a cure. Each discovery leading to a cure is only one bit of knowledge, a stepping-stone over which we must pass.

So what are some steps that might facilitate and augment moving our basic knowledge to translation? We might choose amongst several ways to enhance communication with clinicians and clinical scientists and to gain knowledge in clinical fields. Certainly the basic scientist may reach out directly to the clinical scientist. When FASEB sponsored the Translational and Basic Science Research symposium at the Howard Hughes Medical Institute in 2011 (see www.faseb.org/translationalresearch), one basic scientist described how she had taken that approach. To enhance her knowledge about clinical medicine and her ability to communicate with clinicians she took a year-long sabbatical and shadowed clinicians in their practice. I don't suggest that every basic scientist who strives for translation needs to do what she did, but her experience does emphasize how important it is for basic researchers to find ways to communicate with those who have clinical skills and clinical questions.

Communication can also be enhanced if the basic scientist and the clinician simply talk over lunch. One of my colleagues, who says that a productive laboratory is a laboratory that has lunch together, has a lunch room in his lab to facilitate communications. His is a basic science lab, but his philosophy emphasizes how critical it is for us to connect with others both in our direct field of interest as well as in other fields that may hybridize with our own ideas and create new vigor.

In some cases hybridization can occur within the individual scientist. Translation itself does not represent a one way street any more than any discipline of science stands alone in its quest for discovery. Thus, just as basic science can promote translational studies, clinical observations made by a clinician who happens also to be a basic scientist may themselves spark basic scientific investigation.

While there are times when the basic scientist may initiate translational research, the basic scientist does not have to bear that responsibility alone. Clinical scientists may come to the basic scientist's door with a problem to be solved as occurred for another of the scientists at the FASEB symposium mentioned above. Clinicians came to him because they knew that he might be able to apply his skills to a problem they could not solve. He was willing to hear them and worked to bridge the gap between clinical and basic science. This in turn led to a new direction for his research and to close translational collaborations.

Such basic to translational collaboration does not have to occur within academia. Industry, of course, applies the basic knowledge coming from our laboratories to development of new products. We need to be aware of their needs and that they may be reaching out to us. It is not unusual for industry to contact universities in an effort to learn if there is a faculty expert who might be able to help them with a particular project that they want to address; so working within your own university to develop a system by which they can reach

individuals may allow them to find you. Some universities, such as Harvard through its Catalyst program (see: <http://catalyst.harvard.edu/>), have formalized a system to expedite such exchanges both intramurally and extramurally and serve as good examples of institutions' fertilizing the field of potential discovery.

Identifying Targets for Translation

Having discussed these general considerations for developing your translational research program, I want to focus on the matter of target identification, a critical step in drug development. As an example, I will describe my own path toward translational research. The journey began as my laboratory sought to study brain-heart interactions both in health and disease, but there would have been no path had we not known of critical work that preceded ours.

In 1942 the noted physiologist Walter Cannon published an article entitled "Voodoo Death"³ Cannon investigated the impacts on ordinary people who had poxes, hexes, or curses placed upon them by revered members of their societies. He found cases in which people had been told that they were going to die and, indeed, did die suddenly and without apparent cause. Cannon hypothesized involvement of the hypothalamopituitary adrenal axis. What Cannon observed in primitive societies also occurs in modern civilization where the link between the hypothalamopituitary axis and the heart may be seen both in the stressed, but otherwise normal brain, and in the diseased brain^{4,5}. It is clear that individuals under great stress, particularly stress from which there is no escape, may develop cardiac structural damage, electrocardiographic changes, and arrhythmias that may be fatal.

This association between stress and fatal cardiac events has been powerfully made by studies of people subjected to earthquakes, which led to a greatly increased incidence of cardiac dysfunction and sudden death with or without evidence of preexisting cardiac disease^{6,7,8,9}. Others have studied the association between earthquakes and cardiac compromise in Japan and have found direct correlation between each temblor and a peak of cardiac events¹⁰.

That said, cardiac compromise and even sudden death also may occur with brain damage such as subarachnoid hemorrhage^{11,5}. The central site, if a single one exists, responsible for the fatal cardiac outcome from such central events is unknown. As a clinician I knew this literature before pursuing a basic study that led to one of my Asimov moments. But the march toward those moments was not direct.

Indeed, twists and turns on the way to most discoveries demand that we keep our eyes open as we go around the bends. My own drive along this highway came during our studies of potential neurotransmitters that may be involved in transmission of cardiovascular reflexes through the nucleus tractus solitarii (NTS). The NTS is the central site where baroreceptor afferent nerve fibers, arising from mechanoreceptors in the aortic arch and carotid sinus, terminate in the brain^{12,13,14}.

We have studied the NTS for years, and we have shown that a number of different potential candidates for neurotransmission may contribute to cardiovascular control by the NTS.

Several years ago we began studies of one such putative transmitter, substance P, whose role, if any, in baroreflex transmission was uncertain. We chose to use a toxin to kill neurons expressing the NK1 receptor for SP and thus to eliminate sites for substance P to act in the NTS. By injecting into the NTS stabilized substance P conjugated to the toxic lectin saporin (SSP-SAP; Advanced Targeting, San Diego, CA), we could rid the NTS of neurons with the NK1 receptor (Figure 1). The lesions blunted the baroreflex and concurrently led to profound disruption of cardiovascular homeostasis manifest as marked lability of arterial blood pressure. Such lability has been described in all situations in which the baroreflex has been disrupted centrally or peripherally in experimental animals or man^{15,16,17}. However, there was also a surprise. We found that 25% of treated animals died suddenly a week or more after injection of the toxic conjugate into NTS. We had had no idea the animals were going to die as we had assessed their status at least four times a day postoperatively and had found that they looked normal until their sudden death¹⁸.

Observing this, I then wondered whether our search for discovery in one area may have led us to an animal model of neurogenic sudden death and potentially a model replicating what is seen in humans. To address this question we needed to study our experimental animals while they were instrumented for recording cardiovascular variables as well as electrocardiographic activity. We did that and in the conscious, freely moving animals we found that the saporin conjugate in NTS led to marked lability of arterial pressure. All of us have quite normal fluctuations in pressure. But, comparing the animals that had been treated (Figure 2) with ones that had not (Figure 2), we found far greater fluctuations in pressure in the treated animals.

Other variables were also disturbed in treated animals. The electrocardiogram of treated animals (Figure 3) at first was normal with P waves, QRS complexes, and T waves all in a normal rhythmic pattern. But just prior to the animal's sudden death, the electrocardiogram became abnormal. Often P waves could be less well defined and at times could not be detected. Over the course of about three minutes the electrocardiogram devolved to asystole; and of course the animal died (Figure 3).

Not only did some animals die a cardiac death in asystole, but some of the animals, even those that survived, were found further to mimic the human condition. Namely, they showed evidence of highly excitable ventricular myocardium with frequent single or repetitive ectopic ventricular beats (Figure 4). To date all animals that have died did so in asystole. No animal has died due to ventricular tachycardia or ventricular fibrillation though ectopic ventricular beats certainly provide a setting in which that could occur.

Examining the hearts of treated animals that had labile blood pressures we found distinct abnormalities. Further we found that the abnormalities correlated with lability. More labile blood pressures were associated with a greater likelihood of histologic evidence of coagulation necrosis in the heart¹⁹. As shown in Figure 5 regions of coagulation necrosis were surrounded by normal cardiac myocytes while yet other regions of coagulation necrosis were present in nearby tissue. There was no correlation between the sites of coagulation necrosis and the coronary arteries. These microscopic necrotic areas were seen diffusely throughout the heart in a distribution like that seen in humans⁵.

Returning to our original question regarding stress and sudden death, we wondered, whether there was any possibility of a mechanism's being found in the sympathetic innervation of the heart. Asking (and answering) that question could clearly lead to translation. Were we to find a potential mechanism that might explain the cardiac changes in an animal that had such a discriminating lesion in one place in the brain we could open avenues to reverse any such changes that might have occurred in neurogenic influences on the heart. Therefore, we are currently performing immunohistochemical studies and quantitative protein analyses in the heart and stellate ganglia to determine if the central lesions lead to changes in tyrosine hydroxylase, the rate limiting enzyme for synthesis of the sympathetic transmitter norepinephrine, or changes in neuronal nitric oxide synthase (nNOS), the biosynthetic enzyme responsible for synthesis of nitric oxide by neurons. Given that nitric oxide may modulate sympathetic function in the heart^{20,21} we also seek to determine if upregulation of nNOS in the stellate ganglion might attenuate cardiac events following lesions in the NTS or, conversely, if we could worsen cardiac effects of central lesions by downregulating expression of nNOS. The tack that we are taking is to use the viral vector adeno-associated virus type 2 (AAV2) to transduce signals within the ganglia. We seek to upregulate nNOS expression in the stellate ganglion by introducing AAV2 with the cDNA for nNOS and to down regulate the expression of nNOS within the ganglion by using AAV2 with interference RNA that we've developed to selectively rid the ganglia of nNOS expression. By so doing we hope to tell whether modulating nNOS at the ganglionic level affects (decreasing or increasing) the probability of cardiac events in otherwise normal animals. Answering the question of basic mechanisms responsible for cardiac events with central dysfunction could allow development of interventions to reduce such events in humans at high risk for their development.

The chase continues

Ten years have passed since our first published observation of sudden death in rats with central lesions and we are still working our way toward an understanding of what might be happening. Possible application (translation) to human conditions only arose because the animal studies produced a condition like that seen in humans. In this case, with my being both a clinician and a basic scientist, the communication of clinical questions was internal, i.e. from within the basic scientist who had made the observation. We are still at "That's funny" and haven't yet reached "Eureka" but we hope the road leads to that end. Often retrospective discussion may make it seem that identification of targets was planned and straightforward. As our path has shown, however, one doesn't always see possible targets from the start and their identification may arise only after an observation, a question, further consideration, and exploration of possibilities that may arise. I would argue that myriad targets await us and yet will only come to light through support of those investigator initiated novel studies that themselves may not seem directed toward a cure. We need always to keep in mind the process toward target recognition and examples of the length of the road to discovery whenever we talk with the lay public or elected representatives who rightfully ask us to point out the value-added to the human condition by the billions of dollars spent to support NIH research. Just like the desperate surgeon who longed for his wife's cure we must recognize that translation and treatments are themselves part of the

orderly sequence from basic discovery. We must seek ways to expedite that orderly sequence while not abrogating it.

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Figure 1. Loss of neuronal staining for NK1 receptors (NK1R) in the medial subnuclear region of NTS in a representative animal treated with SSP-SAP (right figure) when compared with dense NK1R-IR in neurons of the same region of NTS in a control animal in which no injection has been made.¹⁹

Figure 2.

Within 4 days of injection of SSP-SAP bilaterally into the NTS animals began to manifest marked lability of mean arterial pressure (MAP; lower tracing) when compared with normal degrees of lability of MAP in control rats (top tracing) into which PBS had been injected bilaterally.¹⁹

Figure 3. Some rats treated with SSP-SAP experienced sudden, unexpected death as a result of rapid evolution of their cardiac rhythm from a normal pattern (top trace) to asystole and death (bottom trace).¹⁹

Figure 4.
Animals treated with SSP-SAP demonstrated a significantly increased incidence of ventricular ectopic beats (seen in the bottom ECG tracing) with concomitant falls in MAP (top trace).

Figure 5.

Animals treated with SSP-SAP often demonstrated diffusely distributed signs of coagulation necrosis seen in individual cardiac myocytes (arrows in low power section on left and in the high power view on the right of the boxed area from the left-hand figure) as seen in this hematoxylin and eosin (H&E) stained section. Affected myocytes were surrounded by histologically normal myocardium. Areas of necrosis did not correlate with coronary arteries. Their presence was correlated with the degree of lability of MAP.¹⁹