FEATURE

The Secret Lives of Neurotrophin Receptors

Neurotrophins are a family of growth factors that are critical to the proper development and functioning of the nervous system. Neurotrophins activate a family of tyrosine receptor kinases (Trk), which typically initiate signaling cascades through phosphorylation. This axis is important for central nervous system (CNS) drug development efforts, ranging from pain management to neurodegeneration. However, neurotrophin-activated pathways are important for a variety of cancers and their metastatic properties. Indeed, TrkA, the prototype of the neurotrophin receptor family, was first identified at NCI as part of a fusion oncogene. Moreover, Trks are widely expressed in many different organs where their misactivation has been associated with tumor formation. Trks are also present as truncated receptor isoforms, lacking kinase activity, and these forms are particularly prominent in adult tissues. Little is known about the role of neurotrophins and Trk receptors outside the nervous system. Lino Tessarollo, Ph.D., Director of CCR's Mouse Cancer Genetics Program, uses his expertise in developing genetically modified mouse models to dissect the functions of these receptors, with the goal of developing insights that will guide the successful targeting of therapeutic interventions.

Imprinting on Neurotrophins

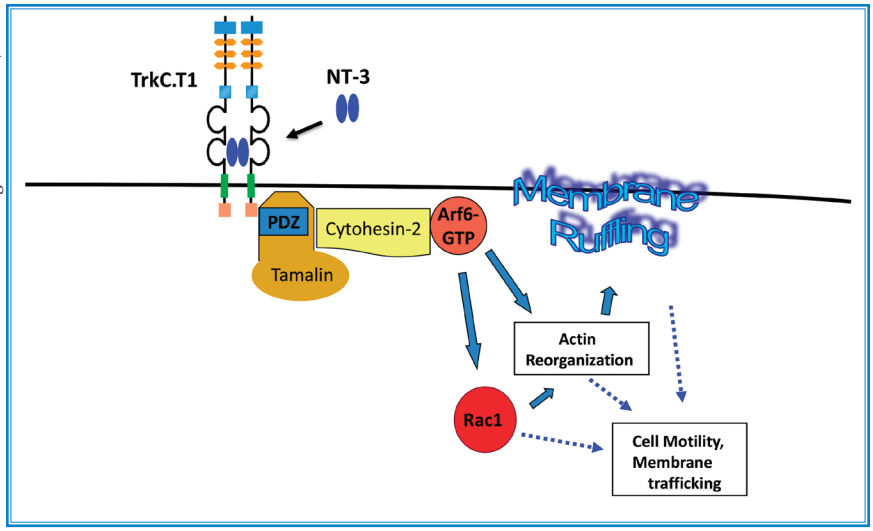
Tessarollo came to the Frederick Cancer Research Facility in Maryland (now known as NCI at Frederick) at a scientific moment that has defined his career. He joined the laboratory of Luis Parada, Ph.D., as a Postdoctoral Fellow in 1990, just a few years after Dionisio Martin-Zanca, Ph.D., Stephen Hughes, Ph.D., and Mariano Barbacid, Ph.D., also working in Frederick, cloned a human fusion oncogene from colon carcinoma cells and discovered one of the first transforming genes in a human malignancy, TrkA. When Tessarollo arrived, Martin-Zanca had just joined Parada's lab and was searching for the ligand that could bind and activate TrkA.

Meanwhile, a young Principal Investigator, just recruited to Frederick, David Kaplan, Ph.D., wanted to identify the proteins that were phosphorylated in PC12 cells in response to nerve growth factor (NGF), thinking that one of them was its receptor. Kaplan and Martin-Zanca soon realized they had something in common: one had a protein around 140–150 kilodaltons in size that was phosphorylated in response to NGF, the other had TrkA, a receptor of about 150 kilodaltons. Their pivotal discovery that NGF is the ligand for TrkA paved the way to an entire field of research into related neurotrophin receptors and their actions, including TrkB and its ligand, brain-derived neurotrophic factor (BDNF), as well as TrkC and its ligand, neurotrophin-3 (NT-3).

"Dionisio was an amazing molecular biologist, who taught me a lot during many long nights at the bench, and David was an amazing biochemist. Without these skills coming together, the finding would have probably taken longer," said Tessarollo. "I was just a spectator in the unfolding story. But it was a very exciting time and I was hooked. Eventually, as the others moved on, I kept the lights on for neurotrophins and Trk receptors at Frederick."

Although the field in general has focused on the role of Trks in the nervous system, Tessarollo knew from the first studies he performed on Trk gene expression that the receptors were found in many different organs outside the nervous system. Moreover, *TrkB* and *TrkC* genes are alternatively

(Figure: L. Tessarollo, CCR)



Truncated Trks signal independently of the Trk tyrosine kinase receptors.

spliced into multiple isoforms, but the two most common are the full-length tyrosine kinase form and a truncated form. The truncated forms are the predominant forms in adult tissues, but their function is almost entirely unknown.

"Basically, what I want to do with my research is to achieve a molecular dissection of Trk signaling," said Tessarollo. "We know that these genes control many different physiological aspects in mammals. If we can dissect how these pathways are activated, then maybe we can generate druggable targets specific for desired effects."

Beyond Development

Tessarollo uses genetically modified mice as his model of choice for understanding the physiological functions of neurotrophins and their receptors. "I like to look at the physiology first. I want to have a phenotype and then try to understand the molecular mechanism," said Tessarollo.

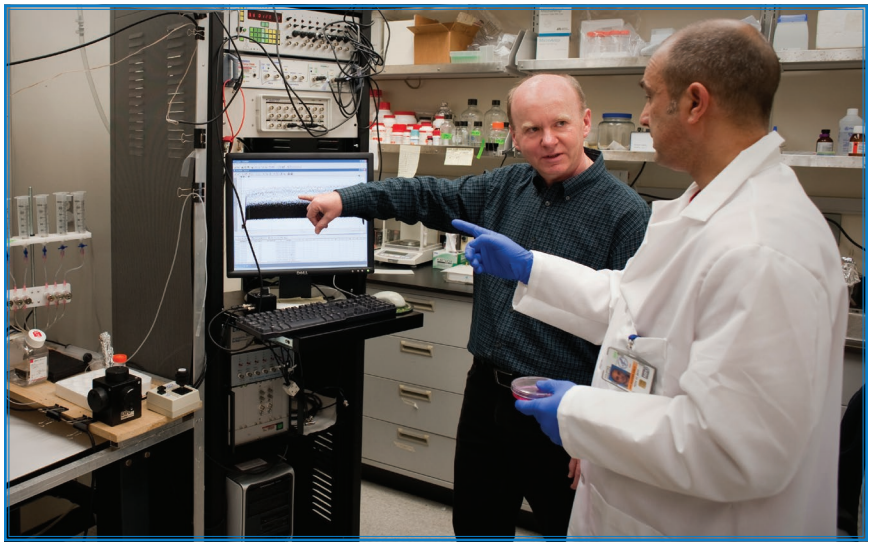
In 1999, Tessarollo and his colleagues published the first evidence that BDNF regulates food intake and obesity in mice. They showed that a single copy of the gene produced subtle physiological alterations that may be more meaningful to human physiology than a simple knock out, which is lethal. These alterations included changes in serotonin neurotransmission and in serotonin-related behavior, such as food intake and aggression. The work presaged later findings of heterozygosity in human *BDNF*. "It really showed that a neurotrophin can be involved, not just in development, but in other aspects of mammalian physiology," said Tessarollo.

As painstaking as mouse model approaches can be, Tessarollo finds this careful work is ultimately rewarding. In 2004, Tessarollo and his colleagues challenged 20 years of literature based on indirect evidence, reporting that NGF was critical for the proper development and functioning of the immune system. They developed a genetically modified mouse model in which they first deleted TrkA and then reintroduced it selectively to cells of the nervous system. This reverse conditional gene targeting strategy generated a mouse with only a very mild disturbance of specific immune cell populations.

"I had a very hard time publishing that paper; it was essentially negative data," said Tessarollo. "But recently at a Gordon Conference, I met a senior pharmaceutical industry scientist who told me that our data was used in their argument to the FDA that an anti-NGF drug would likely not have immune side effects. We worked rigorously to find major immune defects, but simply did not. That turned out to be useful. Sometimes you don't know where science will take you."

Truncated Receptors

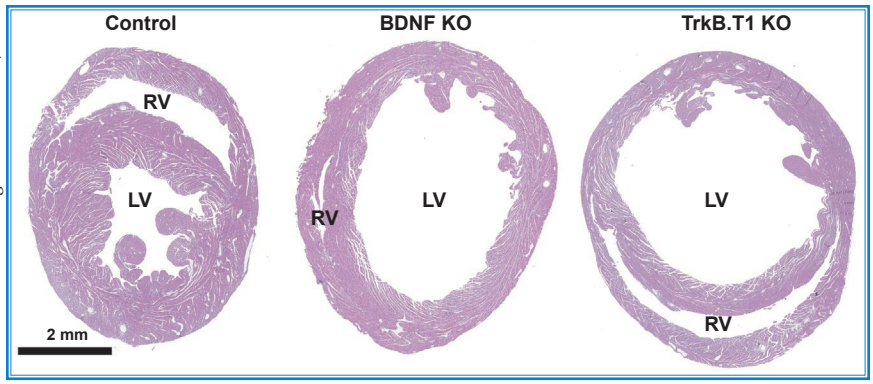
Most recently, Tessarollo and his colleague, Gianluca Fulgenzi, Ph.D., have uncovered an unanticipated



(Photo: R. Baer)

Lino Tessarollo, Ph.D., and Gianluca Fulgenzi, Ph.D., discuss results on cardiac function recordings.

(Figure: L. Tessarollo, CCR)



Deletion of BDNF or its receptor TrkB.T1 in cardiomyocytes causes cardiomyopathy. LV, left ventricle; RV, right ventricle

role for truncated TrkB receptors in cardiac function.

Truncated Trk receptors are difficult to study. The short intracellular tail is extremely conserved across mammals and chickens. Many researchers have tried and failed to find binding partners. So far, Tessarollo has identified a pathway activated by the truncated form of TrkC, but has been unable to create a mouse model to verify the importance of that pathway for technical reasons. Deletion of the exons encoding the truncated isoforms of TrkC resulted in an upregulation of the long, kinase isoform. His laboratory was successful, however, in deleting the truncated isoform of *TrkB*.

"One day we were using a heart as a control for an expression study and discovered that cardiac TrkB receptors are truncated," said Tessarollo. "Gianluca asked me if I had ever looked at the hearts in mice lacking truncated TrkB."

The team discovered that mice lacking truncated TrkB had altered cardiac muscle tissue. This launched them on an investigation of the role of its ligand, BDNF, working through truncated TrkB, on cardiac output. They found that BDNF regulates heart contractile force without involvement of the nervous system. Instead, the truncated TrkB receptor appears to modulate calcium signaling in cardiomyocytes.

"This paper will put the heart on the radar screen of researchers studying therapeutic interventions with neurotrophins," said Tessarollo. "There is a big push in biotech to target TrkB, which is very important for synaptic plasticity and brain function. People are looking for good agonists to ameliorate neurodegeneration. But if the drug is delivered systemically, cardiac toxicity has to be considered."

Sudhirkumar Yanpallewar, M.D., has worked with Tessarollo, first as a Postdoctoral Fellow, now as a Staff Scientist, for 11 years. His work also focuses on truncated Trk receptors. A few years ago, he published a paper in which he deleted both copies of the truncated TrkB receptor from a genetically engineered mouse model of amyotrophic lateral sclerosis (ALS). He showed that ablating

Building Mouse Models

"The reason I landed my first real job is because I learned homologous recombination," said Lino Tessarollo, Ph.D. "It was a very imperfect science at the time, but by pulling a lot of all-nighters, I got the technology working reliably to create genetically modified mouse models."

In 1994, in addition to his own research, Tessarollo set up a Gene Targeting Facility to assist investigators in creating mouse models. Tessarollo works collaboratively with investigators across CCR to develop mouse models of cancer and related systems. For example, he worked with David Levens, M.D., Ph.D., Senior Investigator in CCR's Laboratory of Pathology, on a mouse engineered to express a fluorescent Myc protein upon activation of the *myc* gene, in order to monitor *myc* expression in living cells.

"A great deal of cancer research is done in tissue culture, usually in transformed cells. But, if you want to know what is happening in normal cells, which was key to our work with *myc*, how do you get cells that you are 100 percent sure are normal? To get close to a true physiological state, you need to develop an animal model," said Levens.

Levens consulted with Tessarollo on how to design the recombination vectors to target the gene, and once this work was completed, he handed off the mouse generation and breeding.

"There was both a lot of science and a lot of art to it, and Lino guided us through that. There's no way we could have done this on our own," said Levens. "Lino's expertise is broad and he gets things done quickly. He's got an ability to inform and instruct in a very collegial manner."

> "It's Lino's work *in vivo* that has highlighted the significance of these [Trk] receptors."

the truncated form of the receptor delayed onset of motoneuron degeneration and muscle weakness, suggesting that the truncated form was normally acting to limit BDNF's neutrophic actions through the kinase form of the receptor.

"People always knew the truncated Trk receptors were there, but no one knew what they were doing," said Yanpallewar. "These receptors don't have kinase or other functional domains. It's Lino's work *in vivo* that has highlighted the significance of these receptors."

In the last few years, a new gene editing technology, CRISPR, has taken genetic engineering by storm, making it much easier to make targeted changes in genomic sequences. Using the new technology, Yanpallewar and Tessarollo are looking again at creating a selective knock out of the truncated form of *TrkC*.

A CRISPR Future

"I have always been fascinated by genetic engineering," said Tessarollo. "CRISPR has created a bonanza. It is really amazing."

Recently, using CRISPR technology, his team has made a series of mice by modifying different domains of *Trks* to increase the activity of these receptors. These gain-of-function mutations are typically more difficult to achieve than loss-of-function mutations; they often result in inadvertent inactivation. CRISPR technology speeds up the trial-and-error process. The models will help Tessarollo and his colleagues explore what happens in the nervous system if one is really able to increase neurotrophic function, as people have been trying to do with drug interventions for decades.

These mice cannot only help to determine the therapeutic possibilities of augmenting neurotrophin signaling, they can also address the longstanding question of whether boosting neurotrophin signaling can cause cancer. So far, Tessarollo believes it unlikely. In cancers, when Trk genes undergo mutations that make them constitutively active, they either die or acquire other mutations that allow them to become neoplastic.

"With the type of mice we are now generating, we can address the real utility of therapeutic intervention through neurotrophin signaling," said Tessarollo.

To learn more about Lino Tessarollo's research, please visit his CCR website at https://ccr.cancer.gov/mouse-cancer-genetics-program/lino-tessarollo.

A Mouse-Based Community

Tessarollo's laboratory became part the Mouse Cancer Genetics Program in 1999, under the direction of Neal Copeland, Ph.D., and Nancy Jenkins, Ph.D. He served as Deputy Director of the Program for eight years, and he became Director in 2013. "I was reluctant to take the job, but Bob Wiltrout, CCR's Director at that time, convinced me, and it was exciting," said Tessarollo. "You can make more of a difference for the new generation of scientists. You also have the opportunity to influence the overall direction of the science."

The program is diverse, united in the use of genetically engineered mouse models as a tool for understanding function. The eight additional investigators in the Program have research interests as diverse as cancer stem cells, angiogenesis, epigenetics, and transcriptional regulation.

The emphasis is on basic research, but Tessarollo is always on the lookout for and ready to support translational opportunities. "Shyam Sharan, for example, developed a beautiful system based on embryonic stem cells, in which he can screen hundreds of *BRCA* polymorphisms found in humans whose physiological significance is unknown. He developed that in this program and it has strong translational potential," said Tessarollo. (See "Breast Cancer Genes: When the Sequence Is Not Enough," *CCR connections* Vol. 3, No. 2).

"Often, we generate mice and their phenotypes are not predictable from the functions put forward in the literature for the gene of interest. Having many different areas of expertise around the table can really help people to dissect phenotypes and understand the function of specific genes. The mouse brings us together."