FEATURE

Understanding K-Ras Structure and Function to Develop New Treatments

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Although the K-Ras protein has been known for >30 years and is well understood, researchers have been unable to develop effective therapies for cancers that are Ras driven. Frank McCormick, PhD, Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, California, used his keynote address to present an overview of the structure and function of Ras, emphasizing how this information may be important in developing new cancer treatments.

Ras is responsible for 20% to 30% of human cancers, but Ras cancers are very difficult to treat; therefore, there is considerable interest in understanding the structure and function of the Ras protein in a way that may allow the development of new therapies. As part of this effort, Harold Varmus has begun a Ras program involving 50 people at the Frederick National Laboratory and for which Dr McCormick is serving as the director. Their efforts involve improving understanding of the structure of Ras, related biochemical pathways, and features that distinguish mutated K-Ras from other forms of Ras.

The Ras protein is a heart-shaped protein that moves between 2 different states as it functions to transmit signals from extracellular receptors deep into the cell. It is responsible for cancer development when it becomes fixed in the on position. The major form of Ras in human cancer is called K-Ras.

Efforts have been made to develop treatments for Ras cancers, but have encountered difficulty. For example, Ras controls a wide range of proteins in a series of reactions. Because the interactions are so complex and it is unclear which are critical in Ras-driven cancers, finding one to target with a treatment has been difficult. In addition, Ras binds with very high affinity to nucleotides. Because this affinity is so high, finding a molecule with sufficient affinity to compete with it seemed futile. As a result, that route was not pursued to develop a treatment for Ras-driven cancers.

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Some of these efforts have involved attempts to thoroughly understand the activation and deactivation of Ras. Ras is activated when bound to guanosine triphosphate (GTP). The switch from Ras-guanosine diphosphate (GDP) to Ras-GTP is highly regulated and involves extrinsic mononucleotide exchange factors that bind in response to upstream signals. At that point, Ras can be turned off by negative regulators (GTPase activating proteins [GAPs]), which help Ras hydrolyze GTP back to GDP. When the Ras protein is mutated, it loses the ability to be turned off by GAPs. As a result, Ras is no longer able to respond normally to upstream signals and therapies that target these are not effective for Ras cancers, although they may be effective for some other cancers.

GAPs turn Ras off because there is an active site where GTP is hydrolyzed. When a mutation occurs that changes the active site, binding is unable to occur. Because the site is so small, making it difficult to develop a molecule that could fit, researchers abandoned attempts to fix the malfunctioning site and turned their attention to downstream pathways. However, further studying this to attempt to force the mutant protein to hydrolyze GDP is the first priority of the Ras program at the Frederick National Laboratory.

One GAP that is of particular interest that binds to Ras is neurofibromin (encoded by the gene NF1, which is defective in patients with neurofibromatosis 1 and is also unable to function in many Ras cancers). This gene is present in many organisms, including yeast, but it is not clear what molecule causes it to regulate Ras. The NF1 gene is often mutated in human cancers (including up to 8% of lung adenocarcinomas).

Improved understanding of neurofibromin has come through studying Legius syndrome, a mild form of neurofibromatosis I in which the NF1 gene is wild type. Studying the associated mutation in SPRED1 has provided insight into the functioning of Ras. The wild-type Spred proteins bind to NF1 and recruit it to the plasma membrane, allowing it to turn off Ras. Without these proteins, Ras remains in the on conformation. It may be possible to develop medications that target the specific receptors where this occurs as one way to regulate Ras.

Another primary goal of the Ras group at the Frederick National Laboratory is to determine whether a treatment could be developed to interfere with sites involved in dimerization of Ras. There is experimental evidence that Ras can exist as a dimer and that dimerization is important in signaling. For example, wild-type Ras suppresses mutant Ras, suggesting that Ras may exist as a dimer. If the wild-type Ras binds to mutant Ras, it can interfere with the activity of the mutant Ras.

Because other efforts such as targeting the active site or finding a molecule with high affinity to bind were abandoned, efforts were made to target molecules in the Ras pathway (Raf, Mek, and Erk). Raf inhibitors promote benign tumors that appear rapidly due to the activation of Raf in cells with mutant Ras. When mutant Ras is present, Raf inhibitors activate Raf. Although the pathways are different, similar problems arise when targeting other molecules in the Ras pathway. Inhibiting one molecule in the pathway can activate others, making medications ineffective.

Efforts to target Ras cancers have also focused on different types of Ras. K-Ras is the most common Ras associated with human cancers, and is especially important in pancreatic cancer, colorectal cancer, and lung cancer (95%, 45%, and 35% of which are K-Ras cancers, respectively). By contrast, HRas and NRas look very similar but are less commonly responsible for human cancers. K-Ras acts differently from HRas and NRas because it is capable of inducing a stemness phenotype that increases the ability of K-Ras tumor cells to form tumors, metastasize, and resist medications. K-Ras can bind to calmodulin, affecting other biochemical pathways associated with stemness. When K-Ras is mutated so that it is unable to bind, then stemness disappears.

Efforts to understand the role of K-Ras in producing stemness have led to studies of Leukemia Inhibitory Factor (LIF), a cytokine that is upregulated by K-Ras (which also upregulates other molecules). LIF is responsible for contributing to stemness by maintaining an undifferentiated state. LIF has been studied in human cancers, in which higher concentrations correlate with poorer prognosis. When LIF is knocked down in mouse cells, it interferes with stemness (the ability to initiate tumors and to metastasize). In addition, LIF-neutralizing antibodies interfere with tumor initiation in mice. These findings led to an ongoing experiment in which the following four groups were compared: a monoclonal antibody for LIF, gemcitabine, a combination of the antibody and gemcitabine, and a control group. The combination was extremely effective in eliminating tumors.

Dr McCormick concluded his discussion of these studies by reviewing the current areas of study and noting that "these are reasons to be hopeful that we, as a field, will finally get a grip on these types of cancer and lead to therapies that will benefit patients in the not too distant future."