NRG1 Protein Variant Linked to Schizophrenia

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Schizophrenia is a mental disorder that affects the ability to tell the difference between real and not real. Schizophrenia is not considered to be a personality disorder since it is a chronic disease that requires lifelong treatment. It is one of the many brain diseases found in today population that leads to hallucinations, loss of personality, confusion, and bizarre behavior. Another threatening side effect of schizophrenia is that many develop suicidal thoughts and tend to act upon them. About 3.5 million people in the United States are diagnosed with the brain disorder and develop it at an early age in life. The starting age range of those diagnosed began as early as 18 years old, men tend to start showing symptoms in their early 20’s while women do not start until their late 20’s.

The real cause of schizophrenia has not yet been found, but it can be said that the brain disease is partially genetic\(^1\). A defect in the protein Neuregulin has been associated with the development of the disease schizophrenia. The NRG1 protein is part of a Neuregulin family of proteins that acts on the epidermal growth factor receptor (EGFR) family of receptors. The protein is found in many isoforms and variations that allow it to act out a variety of different functions. The protein along with its many isoforms are composed of an epidermal growth factor binding domain (EGF), which allows for the bioactivity of the protein, and an immunoglobulin-like domain (Ig) or a cysteine-rich domain (CRD)\(^1\). To define these terms more clearly an EGF-like domain is made up of an extracellular protein module that consist of three intradomain disulfide linkages, while an Ig domain is made up of two antiparallel beta-strands arranged into
two beta-sheets. A CRD is composed of about 60 amino acids, which are characterized by multiple cysteine repeats within the pattern. NRG1 is a key protein in the development of multiple organs, nervous system, and the brain. Dysregulation and altered expression of the gene sequence in the different isoforms leads to the cause of diseases, such as schizophrenia.

The different variations and isoforms of the protein are caused by alternative splicing of the mRNA in the NRG1 protein. Alternating splicing occurs during product expression and is the process that results in a single mRNA gene transcribing for more than one protein. The process occurs once DNA has been transcribed into RNA, and RNA is transcribed into mRNA. Once the protein has been transcribed into mRNA alternative splicing occurs creating multiple mRNA products. This leads to the proteins translated from alternative splicing to differ in amino acid sequence, and in their normal function. Alternative splicing occurs at specific splice sites in the gene that are regulated and selected by trans-acting splicing activator and splicing repressor proteins on the pre-mRNA. Some modes of splicing can cause exons to be excluded, included, or retained. Splicing may also cause a donor or acceptor site for the splicing sequences at the 5’ and 3’ cap of the sequence. Alternative splicing allows for the biodiversity of proteins that can be encoded and for multiple gene sequences. In some cases this can be a good thing; however the difference in gene sequence can be implicated in causing genetic disorders. One other mechanism that leads to the formation of the NRG1 isoforms and NRG1 variations is a single nucleotide polymorphism (SNP). SNP allows for a single position in the DNA sequence to be changed. When this occurs the gene is classified as having more than one DNA sequence, usually two no more than three. The genetic differences caused by SNP are suspected to lead to certain genetic diseases, such as schizophrenia.
The exact process and area of the NRG1 protein causing the disease schizophrenia has not yet been pinpointed. Research has developed over the years to find the true relationship between the protein and the disease. All structural parts and components of the protein have been researched and experimented on to get a clearer understanding. The main focus of research has been on the altered gene sequence and signaling activity between the protein and its receptors.

The signal activity by the ErbB receptors in the protein sequence was studied and found to be altered by a protease which initiates a signaling cascade between beta-site APP-cleaving enzyme (BACE1), and ADAM metallopeptides domain 10 (ADAM10) nucleotides. ErbB receptors are composed of tyrosine kinases and hundreds of amino acids. The receptors are involved in the cellular functions of the protein including cell growth, signaling activity, and cell survival. When the signal cascade begins the ADAM10 is located eight nucleotides before BACE1, which is the complete opposite of how they are found in the normal sequence of NRG1. When BACE1 and ADAM10 are in their normal positions in the sequence, they activate the ErbB receptor to normally signal the phosphorylation and myelination of the NRG1 protein. The dysregulation of the nucleotides leads to the release of an N-terminal fragment of the protein that impairs NRG1 protein signaling.

Western blotting was performed to analyze the N-terminal fragment of the protein. Polymerase chain reaction (PCR) and gel electrophoresis was first performed to amplify and separate out the cells of interest. The full length cDNA of the protein was encoded and then PCR primers, specific to the nucleotides BACE1 and ADAM10, were placed in the thermocycler to undergo PCR. After amplification of the nucleotide sequence by PCR, the nucleotide samples were run on an agarose gel plate via electrophoresis to fully separate out the nucleotide sequence.
The agarose gel plate was then “blotted” onto a nitrocellulose membrane with filter paper. The agarose gel product was transferred onto the nitrocellulose membrane and were then cultured with a solution of formaldehyde in phosphate-buffered saline (PBS) for fifteen minutes, and then incubated with a mixture of detergent Triton X-100 in PBS and primary antibodies. The antibodies that were used were MBP, which was specific for myelin staining, and SMI-32. After overnight incubation the cells were rinsed and incubated with secondary antibodies, Alexa Fluor 488 and Alexa Fluor 568, which bind to the DNA and a fluorescent tag. After overnight incubation the fluorescent taq cells were then analyzed by fluorescence microscopy. After analyzing the cells, the N-terminal fragments were shown to cause a weaker signal between the protein and the ErbB4 receptor. The NRG1-ErbB4 pathway is in charge of many neuronal functions and alteration of the pathway leads to errors in neuronal functions.

Further research has been performed on the altered pathway between NRG1 and its ErbB4 receptor. Probing was done on the cell line PC12 from the protein, which is known to contain the ErbB4 receptor\(^3\). The PC12 cell line was culture in a 96 well plate with PBS and incubated overnight. After overnight incubation, the cells were rinsed and cells that expressed resistant to the ErbB4 receptor probes were micro pipetted and incubated with a green fluorescent tag to be visualized via electrophoresis. The cells were separated by electrophoresis and an agarose gel plate. The PC12 cell line was examined by fluorescence imaging for the effects on the NRG1-ErbB4 pathway. The fluorescence images were obtained by a fluorescence microscope, also known as an optical microscope that uses phosphorescence and fluorescence to study the organic properties of the sample. The fluorescent images were compared to fluorescent imaging of the normal pathway, and specific compounds, like the ErbB receptors, which altered
the signaling. This allowed for different pathways to be examined and fixation of locations where signaling was being altered.

The growth factor signaling of the NRG1 protein has also been further researched due to the binding that occurs between the protein and both alpha-vB3 and alpha-6B4 integrin receptors. The binding prevents NRG1 to induce ErbB receptor phosphorylation, leading to altered signaling involved in brain disorder⁴. Binding between alpha-vB3 and alpha-6B4 were analyzed via Western blotting and cell culturing. The cells were cultured in DMEM and were placed in a 96 well binding assay along with a carbonate buffer. The cells were then washed with ice-cold PBS and were then blotted onto a fluoride membrane, and incubated with primary bodies. The cells were then incubated with secondary antibodies and chemiluminescence detection reagents. The luminescent signals on the blot were detected and analyzed by a luminescent image analyzer. The binding of alpha-vB3 and alpha-6B4 was found to weaken the signal that induces ErbB4 phosphorylation and does not allow for ternary complex formation between NRG1, ErbB4, and its integrins.

The dysregulation of NRG1 has also been linked to its isoform type IV. The isoform was only detected in brain tissues and found to be brain-specific. The type IV isoform of NRG1 was found to be expressed at early developmental stages and regulated by a schizophrenia risk-associated functional promoter⁵. The variation in type IV of NRG1 results from SNP and alternative splicing. The 5 end cap of the gene gives rise to the novel type IV isoform, due to SNP which leads to five extra exons and two microsatellites covering the end cap. Out of the five extra exons, one of the single SNP is associated with the excess mRNA levels of NRG1 type IV protein in the brain, which results in a higher risk of having schizophrenia. Along with SNP the NRG1 type IV also undergoes alternative splicing. Splicing occurs between the exons E59 and
E24; leading to two different transcripts of the type 4 protein. The two transcripts also create a premature stop codon exon E127, which gives rise to the type 4 becoming an incomplete and nonfunctional protein, known as a truncated protein. The exclusion of the exons E59 and E24 due to alternative splicing, lead to a termination site in exon E127 causing deletion of regions that are designated for bioactivity within the protein. The NRG1 type IV protein is then generated by multiple alternating promoters, which is a common mechanism for generating protein variants that provide tissue of cell specificity like the type IV isoform. The multiple promoters create transcriptional elements that create new levels of mutations within the protein.

The type IV isoform was further studied by the cloning and characterization of NRG1 which was done by RT-PCR with primer-specific amplifications. The cDNA was denatured, annealed, and then polymerized to form a copy of the targeted DNA sequence. Western Blot was used to further sequence and identify NRG1. NRG1 was placed in a PBS solution before gel electrophoresis, and then blotted on a nitrocellulose membrane which became stained by NRG1, and labeled with an antibody to reveal the protein of interest in the sequence. The type IV isoform variant then went under an in vitro luciferase-reporter assay. An in vitro luciferase assay studies the gene regulation and function of a sample by recording the light emission from the reaction of when luciferase catalyzes the oxidative carboxylation of luciferin. The in vitro demonstrated that the 5 cap promoter region of the type IV had an activity associated with the genetic variation of rs994992, a schizophrenia related genetic variant.

Although much research has been conducted, a cure for schizophrenia has not yet been discovered. As of now only mild treatments are available to those diagnosed with the disease such as medications, therapy, and electroconvulsive therapy. Sadly the treatment that is available is extremely expensive and less than half of the people diagnosed with it are not economically
stable enough to afford it. About fifty percent of people diagnosed with schizophrenia have received no treatment at all for it. As of now none of the current treatments available are associated with the NRG1 protein and the dysregulation of the protein. The more research that is done on the disease and on the NRG1 protein; the greater the odds are of discovering treatment possibilities that can become available for the population. The research that has been conducted so far on the protein and its receptors has led to the idea of discovering new pathways so that the signaling between the two can occur normally. If alternative pathways between the protein and receptor signaling that occurs are discovered, then dysregulation of the NRG1-ErbB pathway can be prevented and the neuronal functions of the protein will not be altered. Further research on the NRG1 protein and its ErbB4 receptors and pathways can not only lead to treatment options, but also to possible preventions of the disease.
List of References


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