Understanding the Structure and Function of Protein Kinase C-epsilon using Site Directed Mutagenesis

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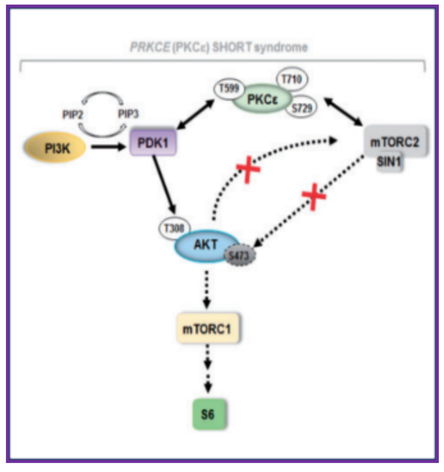
Abstract

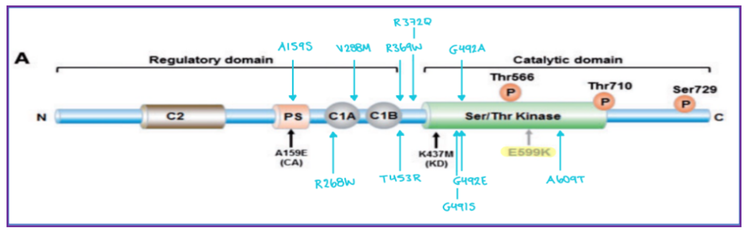
This research is focused on gaining a better understanding of PKC-epsilon a calcium-dependent protein kinase involved in a wide range of cellular functions including cell proliferation, survival, and apoptosis. The interest in PKC-epsilon derives from the discovery of a *de novo* mutation in the PKC-epsilon gene in patients suffering from SHORT syndrome. This syndrome is a debilitating disorder characterized by short stature, hyperextensibility, ocular depression, Rieger anomaly, and teething decay. This project involved recapitulating the naturally occurring *de novo* mutations *in vitro* as well as determining if other mutations in PKC-epsilon could cause similar disease-state phenotypes. Using a technique known as Site Directed Mutagenesis mutations were introduced into the PKC-epsilon gene and the effects of these mutations on the protein expression were assessed. This mutational analysis will help identify the regions of PKC-epsilon that are vital for its function. This will help elucidate the effect of the same mutations in patients and could help correlate with the severity of disease. Obtaining a clearer picture of the different regions of the PKC-epsilon protein allows for future studies to focus on successfully fixing these regions when they become damaged and could therefore be used to help patients with SHORT syndrome.

Background

PKC-epsilon is part of the Protein Kinase C family. It is a kinase that is widely used throughout the cell and can also have implications in cancer presentation as an oncogene. Scientific interest in PKC-epsilon stems from the discovery of *de novo* mutations found to cause the SHORT syndrome physiology. This disease is characterized by different acronym features but can also include other symptoms like insulin resistance and low BMI, patients will usually only experience three or less of the acronym features but there are still many congenital anomalies that impact quality of life. When SHORT syndrome was first discovered it was believed to only be caused by a mutation in the regulatory domain of the Phosphoinositide-3-Kinase Regulatory Subunit 1 (PIK3R1) gene, although a patient was discovered with a mutation in the PKC-epsilon gene adding a new potential disease to the gene. This new mutation established a need for understanding the function of PKC-epsilon in this cellular pathway.



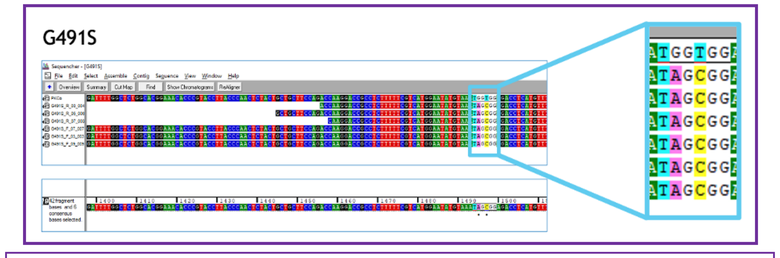
PKC-e SHORT Syndrome Signaling Pathway
SHORT Syndrome was originally believed to only be caused by a mutation in the PI3K gene.
Discovery of a mutation in PKC-epsilon altered the mutated protein in the pathway. Mutated PKC-epsilon causes decreased activation of mTORC2, which can then no longer activate AKT and causes diminished signaling that leads to decreased protein synthesis and can hinder regular cellular functions.



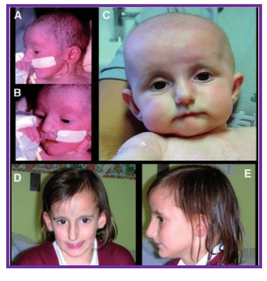
SHORT Syndrome PKC-epsilon Mutations
All the mutations listed in blue were found in patients with SHORT Syndrome. The location of the mutations gives us an idea of how the mutation would affect the function of the protein. These mutations were recapitulated to test the effect on the PKC-epsilon protein in cell culture.

Mutant	Sequence	Sequencing Result	Findings
T453R	CGTGGACTGC**CGG**ATGACAGAGAAG	☑	Only desired mutation
G491S	ATATGTAAAT**AGT**GGAGACCTCATG	☑	Only desired mutation
A609T	TCCCTTTGAG**ACC**GACAATGAGG	☑	Only desired mutation
A159S	GCGGCAGGGG**TCC**GTCAGGCGCA	☑	Only desired mutation
V288M	TGAGACCAAC**ATG**GCTCCCAACT	☑	Only desired mutation
R268W	GGGACTCTTG**TGG**CAGGGTTTGC	☑	Only desired mutation
G492A	TGTAAATGGT**GCA**GACCTCATGTTTC	☑	Only desired mutation
G492E	TGTAAATGGT**GAA**GACCTCATGTTTC	☑	Only desired mutation
E599K	GCTGATGTAC**AAG**ATGATGGCTGGACA	☑	Truncated due to undesired mutation

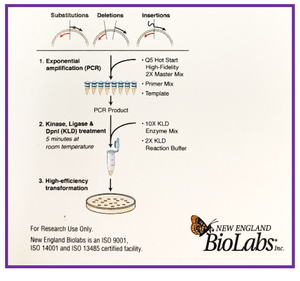
Mutants Generated
All the mutants listed were originally found in different patients with SHORT Syndrome and successfully recapitulated *in vitro*.



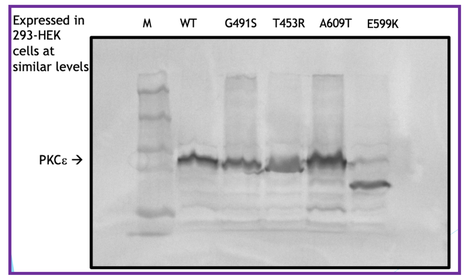
Mutant Sequencing Analysis
Analysis of sequencing reactions was performed with Sequencher software which aligned the different sequences with wild type (WT) and highlighted any differences. In the enlarged portion shown the top line is WT sequence which shows a thymine (T) while all the sequencing reactions show a mutated version with cytosine (C) which was the desired change.



SHORT Syndrome Patient
The patient above shows the typical physical features of SHORT Syndrome such as a triangular face, deep set eyes, and small chin and was the first patient discovered to have a mutation in PKC-epsilon.



Site Directed Mutagenesis (SDM) Protocol
Steps of SDM used to introduce desired genetic mutations into the PKC-epsilon gene.



Expression of Mutants
Western Blot with four PKC-epsilon mutants and wild type (WT). All mutants show similar levels of expression as WT. The E599K mutant ran faster on the gel indicating that it was smaller in size than the other mutant proteins.

Conclusion

Of the eleven mutants that were found in patients, eight of them were successfully recapitulated *in vitro*. The mutants were shown to have similar levels of expression and similar size to wild type (WT). With the exception of E599K, which ran faster and was found to be truncated due to an inadvertent frameshift mutation that was introduced during SDM that required a rework of this mutant.

Further Directions

Overall, this project was the first step in the understanding of the structure and function of the different domains of PKC-epsilon and their role in disease. Continuation of the project will include the determination of the effect of mutations on expression levels and the analysis of the function of PKC-epsilon mutant proteins in mammalian cells. These future steps will help us better understand how different mutations affect the enzymatic activity of PKC-epsilon. Gaining this understanding will assist in correlating the impact on protein function with the severity of SHORT Syndrome disease.

SciCom Summary

English: This project is focused on introducing genetic mutations into DNA in order to better understand the structure and function of a protein, PKC-epsilon which is essential for cell survival. When this protein is mutated or damaged it can cause a disease known as SHORT Syndrome which is characterized by symptoms like insulin resistance and physical abnormalities that negatively impact the quality of life of those affected.

Español: Este proyecto se enfoca en generar cambios genéticos en el ADN para mejor entender la estructura y la funcionalidad de la proteína, PKC-epsilon que es esencial para la supervivencia de la célula. Cuando esta proteína esta mutada o dañada causa una enfermedad conocida como Síndrome SHORT que es caracterizada por síntomas como resistencia a la insulina y anormalidades físicas que impactan la calidad de vida de las personas afectadas de manera negativa.

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