Regulation of DGKι subcellular localization and its effect on actin cytoskeleton reorganization

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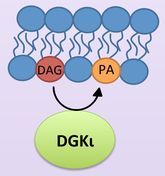
Abstract

The cytoskeleton is a dynamic structure that plays an essential role in cell movement, which is required for normal development and for the metastasis of cancer cells. Diacylglycerol kinases (DGKs), enzymes that phosphorylate the lipid second messenger diacylglycerol to yield phosphatidic acid, regulate actin cytoskeleton rearrangements. The goal of this study is to better understand how one such isoform, DGKι (iota), regulates cytoskeletal rearrangements. Since DGKs function primarily at the plasma membrane, the regulation of their subcellular localization is a key determinant of their activity. To understand how the activity of DGKι is regulated, mutations will be introduced that mimic phosphorylation of the MARCKS domain. Similar mutations in a closely related isoform, DGKζ (zeta), regulate its localization to the plasma membrane. The introduction of these mutations in DGKι should, hypothetically, increase the enzyme's association with the plasma membrane. Analyzing the biochemical and cellular changes that occur as a result of these mutations will provide greater insight to the mechanisms that regulate the actin cytoskeleton and may provide new targets for anti-cancer therapies.

Introduction

Diacylglycerol kinase iota (DGKι) is an enzyme that phosphorylates diacylglycerol (DAG) into phosphatidic acid (PA). This in turn activates a cascade of other biochemical reactions, such as those required to reorganize the actin cytoskeleton.



This process requires that DGKι associates to the cell membrane. In order to increase the association, certain mutations were introduced in the MARCKS domain of DGKι by site directed mutagenesis. Since similar mutations in a related isoform, DGKζ, increased its association with the plasma membrane, it was hypothesized that the mutations will induce a similar effect in DGKι.
The knowledge gained from a better understanding of the processes that regulate rearrangements in the actin cytoskeleton can lead to a multitude of medicinal and pharmaceutical applications, such as the amelioration of treatments for neurodegenerative diseases and cancer.

Goal of the study

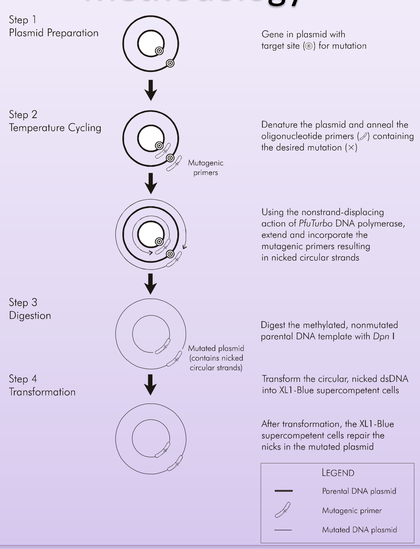
The goal was to introduce a series of mutations to transform the serine amino acids in the MARCKS domain of DGKι into aspartic acid. Phosphorylation of serine causes DGKι to associate to the membrane. By replacing serine with aspartic acid, the negative charge of aspartic acid mimics that of the phosphate group that would be on serine. The introduction of this mutation would, theoretically, cause the protein to behave as though it was permanently phosphorylated. It is hypothesized that this would change DGKι's subcellular location and cause an increase in membrane association.

DGKι MARCKS domain:

Original amino acid sequence: **K A S N R K K K R T S F K R K A S K R**

Introduction of the mutation: **K A D N R K K K R T D F K R K A D K R**

Methodology



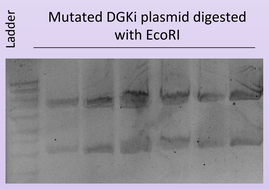
QuikChange® Site-Directed Mutagenesis Kit. Instruction Manual, Revision A.01

Results

Trial	Changes to procedure	Result
1	Original procedure was followed	No colonies grew
2	Added DMSO to PCR reaction and elevated annealing temperature	No colonies grew
3	Changed the buffer	Colonies grew successfully

Gel electrophoresis was performed on trial 3 to ensure that the mutant was indeed successfully cloned.

Since the gel matched up with the correct markers on the ladder, it was determined that the cloning was successful.



Discussion

With a prototype of the DGKι mutant, further experimentation and research can yield a more in-depth understanding of the mechanisms behind actin cytoskeleton rearrangements. Knowledge gained from these experiments can be applied to treatments in neurodegenerative diseases such as Multiple Sclerosis or Alzheimer's disease since it is known that for neurite outgrowth to occur, actin remodeling of the cytoskeleton must take place.

Research on DGKι may additionally provide new applications for anticancer therapies, since tumor metastasis requires cytoskeletal rearrangements in order for cell migration to take place. By studying enzymes that are responsible for cell motility, a wide range of medical and pharmaceutical applications become available.

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