

November 2011

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Recommended Citation


Patel, Mittul (2010) "P40: The Missing Link Between Autophagy and Cancer?," *Kaleidoscope*: Vol. 9, Article 31.
Available at: <https://uknowledge.uky.edu/kaleidoscope/vol9/iss1/31>

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P40: The Missing Link Between Autophagy and Cancer?

Introduction



Autophagy is a lysosomal degradation pathway that is essential to the survival and proper functioning of cells. The process plays a key role in the degradation of damaged cellular organelles, dangerous foreign materials, and proteins. Studies have shown that deletion or mutation of autophagy genes can lead to abnormalities in the cell that could be the root of many devastating human diseases. Of these, cancer has already been shown to be strongly affected by autophagy malfunction. Increased cell growth leads to cell division; uncontrolled cell growth and proliferation can lead to severe abnormalities. The malfunction of autophagy could result in abnormal and uncontrolled cell proliferation and thereby cancer. P40 is a novel protein that may be the missing link between autophagy and the regulation of cell proliferation. P40 interacts with Beclin-1, a key autophagy regulator, as well as γ -tubulin, a protein that is essential to microtubule formation and cell division. This project aims to identify the interaction characteristics of p40, Beclin-1, and γ -tubulin as well as the results of alterations of any one of these proteins and their effects on cell proliferation.

Progress to date

Using PCR it was possible to identify, isolate, and amplify the full-length p40 gene. This was done using primers designed to recognize and allow for polymerization of the full-length p40 gene. This was the first step taken in production of the full-length p40 constructs, which must be properly formed for the success of later parts of the project. Using the isolated and amplified p40 DNA, it was possible to insert the gene into a pEntry-TOPO-D entry vector via a ligation reaction. This construct allows for the ability to use the p40 gene in a variety of applications such as mammalian expression. These vectors were then tested by digestion reactions and PCR as well as sequencing. Once identified as correct, the entry vector was used to perform a ligation reaction with the pcDNA3.1-DEST-47. In order to continue with this vector however, it was necessary to clone a larger volume of it. This was done by transformation into E. Coli cells that were cultured to replicate the desired DNA. The plasmids were then isolated via Maxi Prep, which gives a significant amplification of the vector.

This vector is to be used for transfection and expression in mammalian cells, which will then allow for the testing of various aspects of p40 expression. Meanwhile, the creation of truncated p40 gene construct is underway. Primers needed to conduct the truncations have been designed. These primers will be used to isolate truncated p40 gene and the process of vector creation used for the full length will be used to create truncated gene vectors. The transfection of the p40 expression vector and the isolation of truncated p40 gene to create a vector is the current status of the project.

Next Steps

The full-length construct still needs to be tested on a protein level. This will be done once transfection has been performed by western blotting and a Co-IP has been performed. This will accurately determine the proper expression of p40. This process will be repeated using the truncated p40 gene vectors. Once all vectors are tested, data will be collected via various Co-IP and Western Blot experiments. By analyzing the methods of interaction between p40 and Beclin-1, an important autophagy protein, and γ -tubulin, it will be possible to determine the role p40 plays between these proteins. Furthermore, with continued experiments it will be possible to determine how p40 plays a role in cell proliferation regulation via autophagy, which is the major goal of the project.

References

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