Cloning of Anti-tumor Human Gene SMARCB1/INI1 for the Treatment of Rhabdoid Tumors

Maison Cook, Emma Kempton, Stephen Kotey, Pei Jia Ng, Sean Gile, Judy Hall, Donald Ruhl and Yong Cheng

Department of Biochemistry and Molecular Biology at Oklahoma State University

Abstract

Rhabdoid tumors are malignant tumors found in the kidneys and other soft tissues of adolescents, with the average age of diagnosis between 15 and 24 months. Currently, patients diagnosed with rhabdoid tumors are treated with a combination of surgery, chemotherapy, and radiation therapy. These patients face a survival rate below 25%. The mutations of the SMARCB1/INI1 gene have been found in the majority of rhabdoid tumors including the atypical teratoid rhabdoid tumors (ATRT). The SMARCB1/INI1 protein has the potential to be developed as a tumor suppressing protein-based drug for the treatment of childhood rhabdoid tumors. To test this, we cloned the SMARCB1/INI1 gene from the human genome into a protein expression vector (pET28a). The recombinant human SMARCB1/INI1 protein will be isolated from E.coli cells using Ni-NTA resin-based chromatography and the anti-tumor activity of purified human SMARCB1/INI1 protein will be analyzed in tumor cell culture in vitro in future study.

Introduction

Rhabdoid tumors are malignant tumors that form in the liver, kidneys, and other soft tissues. When they form in the central nervous system, they are referred to as atypical teratoid rhabdoid tumors (ATRT). Rhabdoid tumors are an aggressive form of cancer with low survival rates that are most common in infants and toddlers, with the average age of diagnosis between 15 and 24 months. In individuals with multiple primary tumors, the diagnosis occurs earlier, and they face a worse prognosis. The current treatment consists of a combination of surgery, chemotherapy, and radiation therapy. The gene responsible for the initiation of rhabdoid tumors has been identified as the SMARCB1(INI1/SNF5/BAF47). Cloning this gene is a step toward further analyzing it and would help with further processing to determine its ability to be used as a tumor suppressing drug and increasing the chance of survival in the individuals.

Results

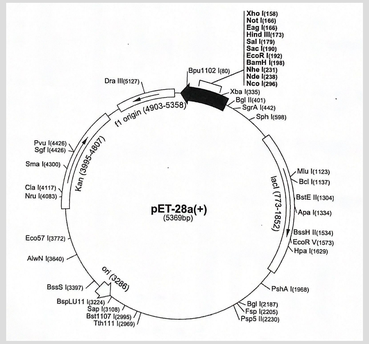

Figure 1: pET28a vector map

Plate #	2	3	4
npH2O	4.0 ul	5.5 ul	6.0 ul
digested gene	2.0 ul	0.5 ul	0.0 ul
pET28a*	2.0 ul	2.0 ul	2.0 ul
10X LB	1.0 ul	1.0 ul	1.0 ul
T4 DNA Ligase	1.0 ul	1.0 ul	1.0 ul

Table 1: Preparation of ligation reaction

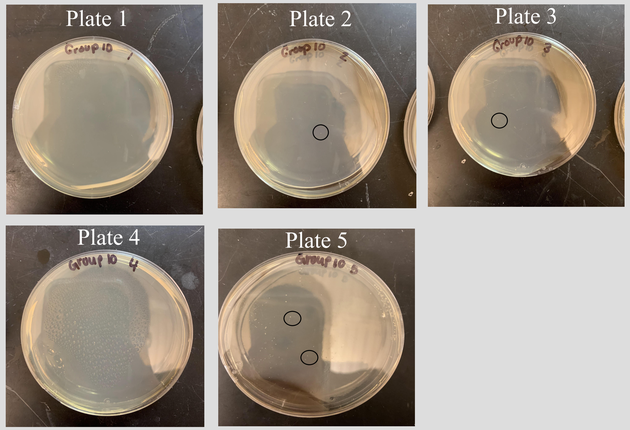

Figure 2: The growth was measured after 24 hours of incubation.
Plate 1: 0 colonies
Plate 2: 1 colony
Plate 3: 3 colonies
Plate 4: 0 colonies
Plate 5: 112 colonies

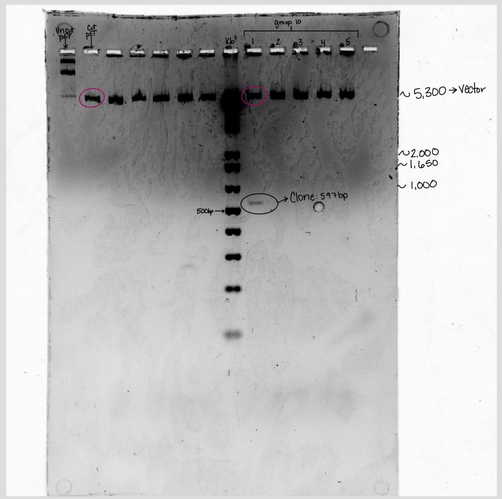

Figure 3: Results from electrophoresis showed a band at 5,300 bp (vector) and one at 597 bp (insert)

Discussion

In Figure 3 the band at 5,300 bp is the vector that we cloned the gene of interest into after digesting the vector and removing the gene of interest. The band at 597 bp shows that we were successful with cloning the SMARCB1/INI1 gene.

Figure 2 shows the growth of *E. coli* on Kanamycin agar plates. The agar is made of an antibiotic that will allow the *E. coli* cells that contain the plasmid to grow, but it will inhibit the growth of the bacterial cells without the desired plasmid. Plates 1-3 show the efficiency of the ligations. A successful ligation will have an increased number of colonies across plates 1-3, with plate 1 having the fewest, and plate 3 having the most. Plate 4 shows the efficiency of the digestions. The lack of colonies shows that complete digestion occurred. Plate 5 shows the efficiency of the transformations, with more efficient transformations having a higher number of colonies.

Conclusion

Using the protein expression vector, we were able to successfully isolate the SMARCB1/INI1 clone that can be seen around 597bps. This isolated clone can be used in medical practice and drug development. The SMARCB1/INI1 protein has the potential to be developed as a tumor suppressing protein-based drug for the treatment of childhood rhabdoid tumors in a similar manner as the technique used in the production of Insulin.

Literature cited

Roberts, C., & Biegel, J. (2009). The role of SMARCB1/INI1 in the development of rhabdoid tumors. Cancer biology & therapy, 8(5), 412-416.

Acknowledgement: Biochemistry and Molecular Biology Course (BIOC3723)