Characterization of Epithelial Growth Factor Transcripts Identified in *Crotalus Atrox* Venom

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Abstract

Epithelial Growth Factor (EGF) is the primary source in regeneration and stimulation of essential fibroblasts cells commonly found in epithelium. It is a polypeptide comprised of 53 amino acids, with most of these amino acids coming from mammalian species. Studies have shown that snake venom components are becoming a growing factor in treating illnesses such as cancer, muscular dystrophy, chronic pain, blood pressure, blood clotting, etc. More specifically, the use of venom from *Crotalus atrox* can be used to treat the further reproduction found in the receptors in EGF. In this study, we cloned and retrieved the transcripts of EGF from the venom of Western Diamondback Rattlesnake (*Crotalus atrox).*

1. Introduction

1. The importance in identifying means of cancer therapy has been a growing priority in recent years. Certain snake venom phospholipases have unique enzymatic properties that enable the wide variety of instruments used to treat the human body. Specifically, *Crotalus atrox* carries a toxin that is known to carry Epithelial Growth Factor (EGF), a protein found in its venom. The EGF found in human cells contains a similar backbone in those found in snake venom. This can propose a means of activating biochemical responses through protein-protein interactions in hopes of regulating unwanted cellular functions.
2. EGF is also found along cellular pathways that promote proliferation, apoptosis, differentiation, and other cellular functions. EGF in snake venom supports promising results in achieving a greater understanding of regulation along cellular pathways through ligands, increasing the likelihood of targeting unwanted cellular growths (cancer), treating cosmetic injuries, bolstering the construction of important pharmaceutical advancements, and much more.

The venom in the Western Diamondback Rattlesnake shows promise in suppressing uncontrollable cell growth (cancer), specifically in EGF receptors

Acknowledgements

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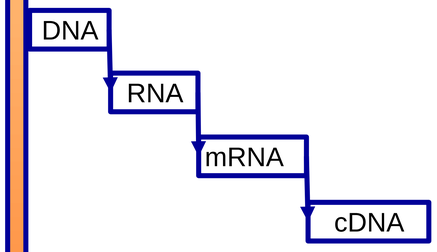
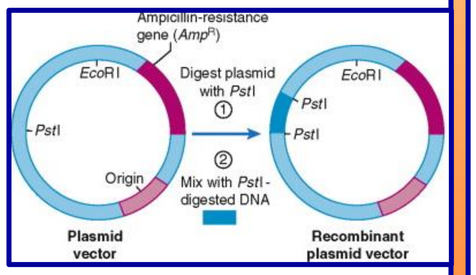
2. We are also grateful for the various organizations such as the National Center for Biotechnology Information in order to produce the models in this project

2. Materials and Methods

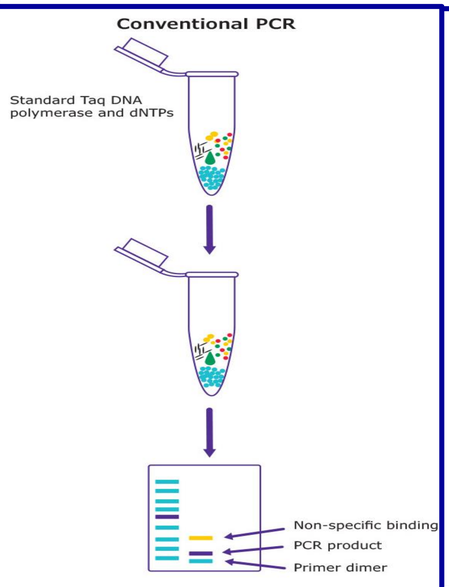
Messenger RNA (mRNA) in crude venom of Western Diamondback Rattlesnake was reverse-transcribed into cDNA. We performed RT-PCR using the EGF specific primers and venom cDNA as template. The amplicons were purified from agarose gel and ligated into a pJET vector.

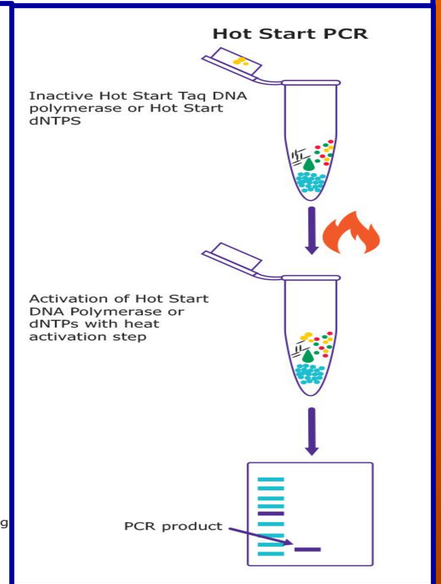




Recombinant DNA was further transformed into bacterial cells, and numerous clones were obtained from colonies.

Unique EFG transcripts were screened based on molecular size and restriction enzyme digestion patterns after isolation of at least 23 plasmid DNAs from bacterial clones.





To identify unique EGF transcripts, we first performed PCR to examine the molecular size of each clones. Clones with same molecular size were further digested by restriction enzyme (*Alu* 1) to see the DNA fingerprints. All DNA molecular sizes and fingerprints were shown on agarose gel (e.g. above pictures).

3. Results and Discussion

Figure 1 shows the successful inserts of EGF transcripts in bacterial vectors that were obtained by PCR. Through gel electrophoresis, we can determine the molecular size of each clone (in kDa) . For instance, the molecular size of clones 12 and 20 are different from the others (upper panel). To distinguish clones with same molecule size, the PCR products were further digested by *Alu* I enzyme (lower panel). Obviously, there are at least three unique EGF transcripts such as clones 1, 4 and 10. The most common EGF transcript is in clone 1 which were further sequenced and characterized.

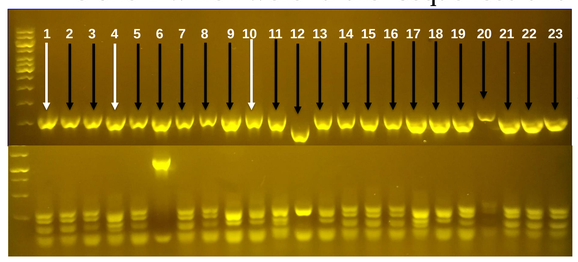
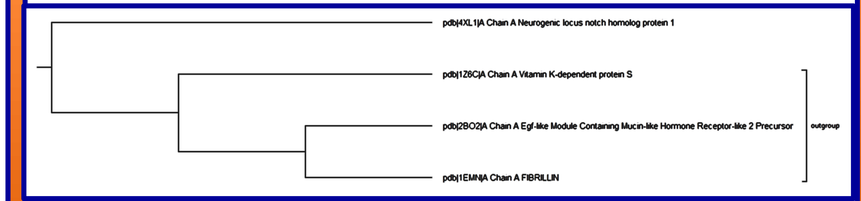
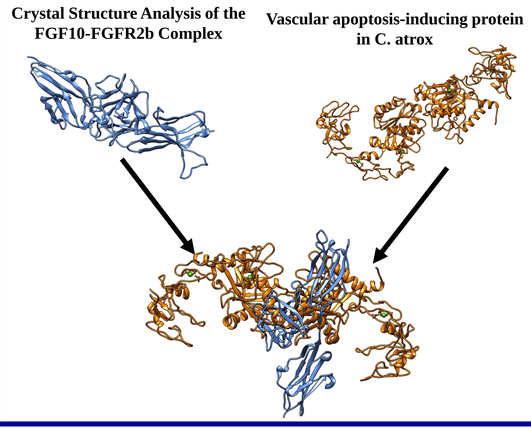


Fig. 1. Screening the unique EGF transcripts from bacterial clones. Gel electrophoresis was used to determine the molecular size and DNA fingerpainters of each clone



The evolutionary history was inferred using the UPGMA method. The optimal tree with the sum of branch length = 2.07988407 is shown. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. This analysis involved 4 amino acid sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 150 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.



The crystal structure of FGF1-FGFR2b Complex is found in Homo Sapiens while the other is found in C. atrox venom. RMSD between 129 atom pairs is 22.504 angstroms when combined. There is a similar structure formation in the backbone found in both proteins. There is a 11.9% identity found in both combined proteins. This modelling is an example of snake phospholipases binding to human EGF proteins in hopes of nullifying phenotypic traits.

Conclusion

1. At least three unique snake venom EGF transcripts were obtained after screening 23 bacterial clones by molecular size and enzyme digestion patterns.
2. The evolutionary relationships and 3D structure of predicted EGF proteins were further analyzed by phylogenetic tree and structure modeling.