شبكة المعلومات الجامعية

بسم الله الرحمن الرحيم

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

قسم

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بعض الوثائق الاصلية تالفة

بالرسالة صفحات لم ترد بالاصل

Use of antisense *in vitro* for inhibition of hepatitis C virus replication

A thesis Submitted to
Chemistry Department
Faculty of Science
Cairo University

For the degree of Master Of Science (in Biochemistry).

Submitted By

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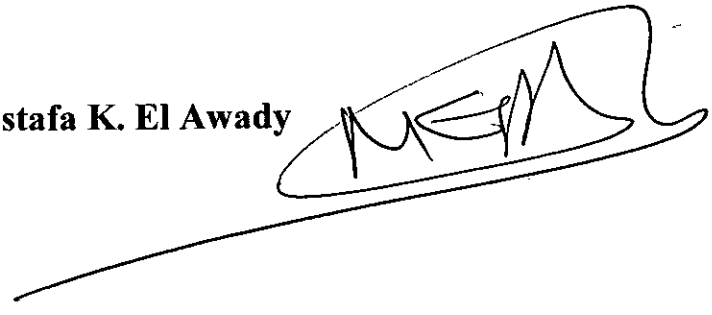

ACKNOWLEDGEMENTS

First and foremost thanks for **ALLAH**

Several outstanding individuals have contributed to making this journey unforgettable and it is to all of them that I feel deeply indebted:

I would like to express my thanks and gratitude to **Prof. Dr. Mostafa Kamel El-Awady** head of Biomedical Technology Department Genetic Engineering Division, National Research Center for suggesting the point of this thesis, his confidence in allowing me to follow my curiosity, determination ,and instincts towards columniation of my research goals. Also, I thank him for his kind supervision continuous support and valuable guidance in all of the theoretical and practical aspects of this work. I am very lucky to have great opportunity to be one of his students.

I would like to express my thanks and gratitude to **Dr. Amr Saad Mohamed** Associate Prof. of Biochemistry , Chemistry Department Faculty of Science Cairo University for his kind supervision, guidance, encouragement and his endless help & support greatly appreciated.

Sincere thanks & gratitude are to **Dr. Mohamed A. El Desouky** lecturer of Biochemistry, Chemistry Department Faculty of Science Cairo University for his keen supervision, revision, guidance and encouragement. His sincere help and endless support greatly appreciated.

I would like to express my thanks and gratitude to **Dr.Samar Sameer Youssef** Assistant Prof. of Biochemistry at Biomedical Technology Department, National Research Center for continuous help and endless support throughout this work. **Dr.Samar** helped me in practical and writing of this thesis. I am also thankful for her generous encouragement despite the many failure science has to offer .

Many thanks are to **Dr.Moataza Omran** Assistant Prof. of Biochemistry at Biomedical Technology Department, National Research Center for her kind assistance and endless support.also I have been lucky to work with a great deal of amazing and talented individuals. I am deeply endebted to Dr.**Sherif Shawky** for his continuous help and kind co-operation and endless support. Many endless thanks to all members in the Biomedical Technology Department who helped me directly or indirectly.

Abstract

Introduction: Although interferon and ribavirin combined therapy is the only approved HCV therapy, this therapy is costly, prolonged, and is associated with significant adverse effects. Furthermore; its outcome is unfortunately poor with genotype 4. Development of alternative therapy for this genotype is of a paramount importance. Inhibitions of HCV gene expression in vitro by the use of antisense phosphorothioate oligodeoxynucleotides (S-ODN) against internal ribosome entry site (IRES) elements were associated with favorable results. **Methods:** To assess S-ODN activity, IRES domain III derived from nine Egyptian patients infected with genotype 4a were amplified, cloned and sequenced. In addition, IRES domain IV sequences that belongs to another group of patients infected with genotype 4a that were previously performed in our laboratory were obtained from GenBank. Alignment of both domains revealed that domain IV is highly conserved over loop III d which showed less sequence conservation. Such conservation suggests higher efficiency of S-ODN1 (directed against domain IV) than S-ODN2 (directed against loop III d). Effect of mismatched oligonucleotides on intracellular HCV RNA levels was also studied by using S-ODN1 altered sequence (S-ODN1*) after random introduction of a single nucleotide substitution which showed no significant effect on S-ODN1 inhibitory effect on viral replication. The efficiency of S-ODN1 to inhibit viral replication in two different cell types was then investigated using HepG2 cells and PBMCs. **Results**: The current study have shown that SODN1 was efficiently able to inhibit viral replication in infected HepG2 cells while; in contrast, it failed to inhibit viral replication in PBMCs. **Conclusion:** The antisense oligonucleotides displayed differential inhibitory effects in different types of HCV permissible cells suggesting that S-ODN1 may inhibit HCV replication via cell specific mechanisms (pathways).

Key words:
HCV, S-ODN, IRES, alignment, HepG2, PBMCs, viral replication

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