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Abstract Title: Antisense Oligomers Based Approaches for Controlling Candida albicans Filamentation
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Abstract:
Introduction: The increase of multidrug resistance of Candida pathogenic species to the traditional antifungal agents and the scarcity of new classes of antifungal drugs led to the increased of the number of infections caused by Candida albicans . One of the most relevant virulence factors attributed to C. albicans is their ability to switch from yeast to filamentous forms. These factors constitute a clinical problem, resulting in high morbidity and mortality, as well as, an economic burden associated with prolonged patients´ hospital stay. Thus, new strategies to manage Candida infections, with novel mechanisms of action and less toxicity, are urgently needed. Antisense therapy (AST) holds great promise for the treatment of genetic human diseases and can be a potent approach to control the complex network of the genes adjacent to virulence factors of Candida species (such as, HWP1, EFG1, HWP2 and CSA1). Thus, our main aim is to design gene-specific oligomers that could target each of these regulators and thus block their molecular function. In addition, this work intends to generate a cocktail of antisense oligomers and validate its in vitro application in order to control C. albicans filamentation.
Methods: Two specific antisense oligomers against EFG1 and HWP1 were designed and synthesized, based on 2'-O-methyl chemical modification through bioinformatic tools. Fluorescence in situ hybridization (FISH) assays were used to infer about the capability of the ASOs to penetrate Candida cell walls and to determine its sensibility and specificity. Candida albicans cells were treated with the ASOs and the % of cells filamentation reduction was enumerated by microscopic observations. The ASOs effect on the genes level expression and on proteins translations inhibition was evaluated by RT-PCR and mass spectrometry analysis, respectively. The ASOs cytotoxicity against human cells was also evaluated by MTS assay.
Results: FISH assays demonstrated that ASOs were able to penetrate Candida cell walls with high sensitivity and specificity. Moreover, the results revealed that C. albicans cells treated with 40 nM of ASOs presented approximately 15% of reduction in their filamentation. Furthermore, the molecular tools were demonstrative of an inhibition of proteins translation coupled with approximately 80% of reduction in the levels of EFG1 and HWP1 expression after 6h of C. albicans treatment with the respective ASOs. It is important to address that no cytotoxicity effects were observed for ASOs concentrations below 40 nm.
Conclusions: Undeniably, this work provides potentially valuable information for future research into the management of Candida infections in order to develop a credible and alternative method to control C. albicans infections, based on AST methodology.