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Gene silencing of Thymidylate Synthase in prostate cancer cells using Polypurine Reverse Hoogsteen Hairpins

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The Thymidylate synthase enzyme (TYMS) is used as an anti-cancer target because of its role in the synthesis the novo of dTTP and it is known to be autoregulated at the translational level. Treatment with TYMS inhibitors (e.g. 5-FU) can lead to tumor resistance through overexpression of TYMS, either by chromosome aberrations or by alteration of molecular mechanisms that regulate gene expression of this gene. It has been proposed that G-quadruplex nucleic acid sequence motifs may regulate translation as well as transcription. Therefore, we explored the incidence of G-quadruplex motifs in the 5' untranslated region (5'-UTR) of the mRNA of the thymidylate synthase gene as potential targets in cancer treatment. By using the QGRS mapper, we found a predicted G-quadruplex (G4) that was confirmed by circular dichroism and UV melting measurements for the RNA sequence. In consequence, and to develop new therapeutic agents to TYMS inhibitors, we designed a Polypurine Reverse Hoogsteen hairpin (HpTYMS-G4-T-PPRH) against the complementary strand of this G4 sequence.

First, we demonstrated the ability of binding of the PPRH to its target sequence by gel-shift assays. HpTYMS-G4-T was able to bind to the template strand of the TS ds DNA target sequence in *in vitro* assays. In addition, TYMS either purified or from nuclear extracts was able to bind to this target sequence, both as dsDNA or ssDNA sequences, whereas 2 negative control proteins (DHFR and BSA) did not produce any binding. Additionally, by using the PPRH HpTYMS-G4-T and the purified TYMS we observed that both compete with each other for the binding to the target sequence in the DNA.

Next, as TS is involved in dTTP synthesis, we compared the effect of this PPRH in PC3 cells incubated either in the presence or the absence of thymidine. The HpTYMS-G4-T PPRH was cytotoxic in both media, decreasing cell viability to less than 5% for at a concentration of 100 nM. These results raised the concept that TS could be involved in other proliferation pathways. The response of PC3 cells after treatment with HpTYMS-G4-T was dose dependent. This PPRH could be altering the transcription of TS gene at this site or the regulation of a G4 structure at this point. In fact, TYMS mRNA determination by RT-qPCR after incubation with HpTYMS-G4-T confirmed that the mRNA levels for this protein were decreased. In conclusion, our results show the ability of the designed PPRH to bind to its target sequence of TS and to decrease PC3 cells viability. Therefore, PPRHs can be considered as a new type of molecules to modulate TS expression and overcome the resistance produced by traditional treatments.

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Acknowledgments

Supported by grant RTI2018-093901-B-I00 from Plan Nacional de Investigación Científica (Spain). Quality Mention 2017-SGR-94. AJF, EA and VG are awarded with fellowships from Ministerio de Educación (FPU), Generalitat de Catalunya (FI), and META-CAN Marie Skłodowska-Curie, respectively.