Role of G4-DNA in transcription regulation: new strategies to inhibit gene expression

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We have recently discovered that the control region of the *KRAS* proto-oncogene contains a nuclease hypersensitive element (NHE) that can assume a G-quadruplex structure: a folded DNA conformation stabilized by arrays of four guanines linked each other by Hoogsteen hydrogen bonds (NAR 2006).

NHE, which is conserved both in human and mouse, is located upstream of the main transcription start site and controls most of the transcriptional activity of *KRAS*. The purine rich strand of NHE forms a very stable G-quadruplex structure that is able to arrest DNA polymerase I in primer extension experiments. In the context of transcription, a G-quadruplex at NHE behaves as a repressor element. In fact, specific G-to-A or G-to-T mutations in the promoter that abolishes the G-quadruplex structure, results in an increase of the basal transcription activity, up to 4 times compared to the transcription of the wild type promoter. Moreover, the basal *KRAS* transcription is Panc-1 cells is strongly repressed when they are treated with TMPyP4, a cationic porphyrin that stabilizes G-quadruplex DNA, but not with TMPyP2 which does not bind to quadruplex DNA.

Combining pull-down experiments and mass spectrometry we have identified three proteins binding specifically to the critical G-rich element of *KRAS*: PARP-1, Ku70 and hnRNPA1 (NAR 2008). Proteins hnRNPA1 and its derivative UP1 have been expressed in bacteria and purify by affinity chromatography. Their interactions with the *KRAS* G-quadruplex have been studied by EMSA and FRET. We interestingly found that hnRNPA1 and UP1 efficiently unfold the G-quadruplex of *KRAS*, suggesting that these proteins facilitate the quadruplex-to-duplex interconversion at NHE. Together, the data suggest that NHE exists in equilibrium between a folded, transcription inactive form and a linear, transcription active form. This model for transcription regulation allows us to design new therapeutic strategies to down-regulate the expression of *KRAS* in cancer cells.

We used oligonucleotides mimicking the *KRAS* quadruplex (G4 decoys), that are able to compete the DNA-protein complexes formed at NHE, as decoy molecules specific for this gene. We found that the G4 decoys induced in pancreatic cancer cells a strong antiproliferative effect, with IC$_{50}$ in the order of 500 nM.

The other target under investigation is the G-quadruplex extruded by NHE. Using screening and molecular assays we are testing a number of molecules that stabilize specifically this G-quadruplex. The molecules that repress the transcription of *KRAS* may be used to sensitize pancreatic cancer cells to chemotherapy.