Selective RNA Versus DNA G-Quadruplex Targeting by In Situ Click Chemistry

Raphaël Rodriguez
Centre de Recherche de Gif
Institut de Chimie des Substances Naturelles
CNRS, 91198 Gif-sur-Yvette - France
raphael.rodriguez@cnrs.fr

The identification of novel cancer therapeutics is an intensive area of research that requires the intervention of chemists, biologists and clinicians. G-quadruplex nucleic acids are non-Watson-Crick secondary structures arising from the folding of a single DNA or RNA strand that comprises stretches of tandem guanines. Sequences with a propensity to form these structures have been computationally identified in particular regions of the human genome including telomeres and promoters with a high proportion in oncogenes and tumor suppressors. Such putative G-quadruplex sequences (PQS) are also highly conserved throughout evolution suggesting functional importance for these sequences. Several studies have suggested these structures to be functional in a myriad of biological processes, including telomere maintenance, DNA replication and transcription, splicing and translation. However, their existence in vivo has remained a matter of controversy. Two questions may be formulated independently as 1) do G-quadruplexes exist in cells and if so, what is their biological significance? and 2) can these motifs be selectively targeted by small molecules to modulate their function(s)? To this end, we have observed that the highly selective G-quadruplex-binding compound pyridostatin (PDS) induces DNA damage at non-telomeric regions of the genome in a panel of human cancer cells. Here, we show that pyridostatin promotes growth arrest in human cancer cells via inducing DNA damage in both replication-dependent and transcription-dependent manners. By using chromatin immunoprecipitation sequence (ChIP-Seq) analysis of the surrogate DNA damage marker γH2AX to establish the genome-wide distribution of pyridostatin-induced sites of damage, we demonstrate that pyridostatin targets gene bodies that contain clusters of sequences with a propensity to adopt a G-quadruplex conformation. Importantly, cellular chemical labeling of PDS revealed that the drug targets genomic loci associated with the human helicase hPif1 known to bind to, and to unfold G-quadruplex structures in vitro, thus providing evidence for their existence in cells. In line with these findings, chemical labeling also revealed the association of pyridostatin with the splicing factor SC35 implicating transcription and RNA G-quadruplex structures (unpublished results). We also show that pyridostatin modulates the expression of targeted genes, which include the proto-oncogene SRC. Finally, we demonstrate that pyridostatin reduces SRC protein levels and diminishes SRC-dependent cellular motility in a human breast cancer cell line, hence validating SRC as a target for pyridostatin. This unbiased approach to define genomic sites of action for a small molecule establishes a framework for discovering functional DNA-drug interactions [1]. The identification of functional DNA and perhaps RNA G-quadruplex structures prompted us to synthesize an RNA-selective G-quadruplex targeting small molecule to interrogate the cellular involvement or RNA-mediated processes in the phenotype induced by pyridostatin analogues. To this end, we have employed an unbiased approach based on in situ click chemistry [2].
