

Pioneering structural and functional studies of miRNA G-quadruplexes

miRNAs are short non-protein coding RNAs, being though a crucial regulators of gene expression of up to 90% of human genes. These tiny RNA molecules tune cell growth, tissue differentiation, cell proliferation, embryonic development, apoptosis and cellular signaling. Even slight shift in miRNA level could lead to significant changes of transcriptome, and in a result of cell phenotype. In the last decade, over 2500 human mature miRNA sequences were deposited in miRBase. The function of many of them have been found and anti-miRNA, as potential therapy tools have been designed. Despite of an enormous data of miRNA, there are still many questions concerning miRNA function to be solved. The generally accepted model of the miRNA-guided RNA down-regulation suggests that mature miRNA targets mRNA in a nucleotide sequence-specific manner. However this model does not take into account a level of miRNA expression, concentration and thermodynamic. We have shown that the nucleotide sequence of miRNA is not the only determinant of miRNA specificity. Following Francis Crick's famous statement "If you want to understand function, study structure", we are looking for structure of mature miRNAs (Belter et al., 2015).

The aim of the proposal is to show the structural diversity of miRNAs. Previously, we have shown that miRNAs can adopt hairpin and/or homoduplex structures (Belter et al., 2015, Rolle et al, 2016). Now we would like to be the first ones who give evidences that some miRNAs may adopt quadruplex structures, which may determine their function.

Till now, we identified guanosine-rich miRNAs and found that the level of G in some miRNAs is even over 90% and noticed that some miRNAs have quadruplex specific motif GGN(1-7)GGN(1-7)GGN(1-7)GG. Using specific nucleases, UV/Vis and CD spectroscopies, small-angle X-ray solution scattering (SAXS) as well as and molecular dynamics, we will give evidences that some miRNAs may adopt quadruplexes in solution. Going further, we will visualize miRNAs quadruplexes in the cells and will try to determine functional consequences of miRNA folding into quadruplexes.

We think that the knowledge of the miRNA structure and its dynamic may give a new insight into miRNA-dependent gene regulation mechanism and be a step forward in the understanding their function and involvement in cancerogenesis. Furthermore, this may improve design process of anti-miRNA therapeutics. The structure of miRNA has functional consequences and suggested that miRNAs may function also beyond RISC and are even more sophisticated regulators, that it was previously expected.

Belter A, Gudanis D, Rolle K, Piwecka M, Gdaniec Z, Naskręt-Barciszewska MZ, Barciszewski J (2015) Mature miRNA form secondary structure which suggests their function beyond RISC, PLOS One, 9, e113848.

Rolle K, Piwecka M, Belter A, Wawrzyniak D, Jeleniewicz J, Naskręt-Barciszewska MZ, Barciszewski J (2016) The sequence and structure determine the function of mature human miRNAs, PLOS One, 11, e0151246.