ABSTRACT

Stem cell residing within the brain can be recognized as a niche that is situated at the intersection between normal brain's differentiation and its malignant transformation that leads to the rise of deadly brain cancer – glioblastoma. This notion prompted the idea that directing cancer stem cell (CSC) fate toward differentiation will dampen their aggressiveness.

One of the novel strategies to push CSC toward the therapeutical differentiation path is the replacement of multiple microRNAs that are responsible for imposing terminal differentiation and are frequently lost in cancer cells, including glioblastoma. This proposal is based on the premise based on our 10-years research that the restoration of multiple microRNAs lost in glioblastoma faces considerable obstacles concerning dosing, stability, effectiveness of the delivery, and possibility of off-targeting; thus to simultaneously deliver multiple microRNA molecules, we propose to get to the juncture of the problem by demystifying the reason why these microRNAs are lost at the first place. In a broad sense, we aim to fix the underlying cause of the problem, rather than to deal with its effects.

In our preliminary efforts, we inspected which parts of microRNA processing machinery may be broken, and we concluded that the process is malfunctioning at the crucial transition from precursor to mature microRNA. We discovered previously unknown non-coding RNA molecule – *circ2082*, that inhibits enzymatic protein complex responsible for such transition. We thus hypothesized that knockdown of *circ2082* would restore microRNA processing in CSCs.

Circ2082 belongs to the recently discovered family of circular RNAs and, as the name implies, emerges from linear RNA as a circularized molecule with no free ends. As a result, the circularization event creates a unique sequence. These features are crucial as they allow designing the short antisense *circ2082*-inhibitor with no homology to linear RNAs (thus having few, if any, off-targets) while being short and remarkably stable. We demonstrated that the *circ2082*-inhibitor of our design could be efficiently delivered both in vitro and in vivo to CSCs to knockdown *circ2082*. What's most important, such intervention led to un-blocking of microRNA processing in these cells.

RNA molecules are currently increasingly proposed as a promising approach not only in oncology, as either therapeutic targets or as factors improving the specificity of targeting. Having a unique capability of un-blocking microRNA processing machinery in CSCs, our primary goal is to facilitate its clinical deployment against glioblastoma. Thus here we propose a better characterization of the effect of *circ2082* knockdown at the cellular level for the un-blocking of CSCs' differentiation potential in vitro (aim 1) and in CSC-originated tumors (aim 2), as well as on the molecular level, to unravel the mechanism of cell-fate determination (aim 3). So, if we can redirect cancer cell fate *via* the restoration of microRNAome that is disrupted during malignant transformation, such directional "correction" of microRNAome would thus provide a potent tool for tilting the scale toward differentiation in the glioblastoma CSC, an effective changeover from the therapy-resistant undifferentiated cell into therapy-prone differentiated one.