MIR-139-5P AND MIR-455-5P AS POTENTIAL NOVEL BIOMARKERS IN GASTROINTESTINAL STROMAL TUMOURS (GIST)

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Gastrointestinal stromal tumour (GIST) are the most common mesenchymal tumour of the gastrointestinal tract. 85-90% of the cases are characterized by gain of function mutations in KIT or PDGFRA genes that promote tumorigenesis and cancer progression. The remaining cases do not harbour any alteration in these genes and are indicate as wild type (WT) GIST. KIT/PDGFRA mutant and WT GIST show different characteristics in terms of gene expression and SNP profiles as well as prognosis and therapy response, however some aspects have not been adequately deepened (Corless CL et al, 2004; Hitota et al, 1998).

In the last years, microRNAs (miRNAs), endogenous non-coding RNAs, approximately 18-24 nucleotide (nt) in lenght, have emerged as crucial regulators of gene expression at post-transcriptional level. miRNAs negatively regulate gene expression by base pairing to 3'UTR regions of specific target mRNA genes. miRNAs have been involved in many critical cell processes among which differentiation, proliferation, and apoptosis. Besides this, recently, increasing number of miRNAs have been reported as involved in the development and progression of diseases, including cancers (Kasinski AL et al, 2011; Di Leva G et al, 2011; Garzon R et al, 2009)

Recently, the deregulation of miR-139-5p, miR-455-5p and let-7b in GIST has been proposed as responsible of IGF1R overexpression (Pantaleo MA et al, 2016). To corroborate this hypothesis, we firstly analysed their expression level in a cohort of 36 GIST sample, of which 27 were KIT/PDGFRA mutant and 9 were WT GIST. After a Bonferroni correction miR-139-5p and 455-5p show significant differences between mutant and WT GIST ($p= 0.0003$ and $p= 0.0055$, respectively). Next, we performed a luciferase assay to test if the IGF1R 3'UTR could be a target of these two miRNAs. The 3'UTR has two complementary binding sites for each miRNA. We cloned a region of 2050 bp of the 3'UTR in a vector pMiRNanoGlo (Promega). This system is specifically designed to quantitatively evaluate miRNA activity by the insertion of miRNA target sites downstream of the renilla luciferase gene; reduced renilla luciferase expression indicates the binding of endogenous or introduced miRNAs to the cloned miRNA target sequence.

We transfected two GIST cell lines (GIST48 and GIST882) with this construct in the presence or not of 50nM of exogenous miR-139-5p and 455-5p. For both the lines, we observed a luminescence reduction in presence of the miRNAs, indicating a possible interaction miRNA-mRNA. To further investigate the role of miR-139-5p and 455-5p, we transfected GIST48 and GIST882 cells with miR-139-5p inhibitor or miR-139-5p inhibitor negative control, at 20nM, 50nM and 100 nM. Western-blotting showed the miR-139-5p inhibition led to IGFR1 expression.

Next, we analysed apoptosis and cell cycle through cytofluorimetry, but we could not observed any differences. Finally, we evaluated the cell migration using radius assay. Migration assays measured the movement of cells into an initially cell-free area. In the presence of miR-139-5p

inhibitor cells showed higher migratory activity, recovering completely the gap; the effect was evident in both cell lines, however was more prominent in GIST48. Taken together, our results demonstrated that miR-139-5p plays a pivotal role in GIST through regulation of IGF1R and inhibiting cell proliferation. These preliminary results showed a potential role of miR-139-5p as tumor suppressor and it could be a novel pharmacological target in GIST.

References

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