Integrating miRNA and gene expression profiling analysis revealed regulatory networks in GIST

G. Ravegnini[1], M. Nannini[2], A. Astolfi[3], V. Simeon[4], M. Urbini[3], G. Biasco[2], M. Pantaleo[2], S. Angelini[1], P. Hrelia[1]

1. Department of Pharmacy and Biotechnology, Via Irnerio 48, 40126 Bologna, Italy; 2.Department of Specialized, Experimental and Diagnostic Medicine, Sant'Orsola-Malpighi Hospital, University of Bologna, Via Massarenti 9, 40138 Bologna, Italy; 3. Giorgio Prodi' Cancer Research Center, University of Bologna, Bologna, Italy; 4. Laboratory of Pre-Clinical and Translational Research, IRCCS-CROB, Referral Cancer Center of Basilicata, Rionero in Vulture (PZ), Italy

Gastrointestinal stromal tumors (GIST) are rare disease of the gastrointestinal tract characterized by *KIT/PDGFRA* oncogenic mutations (75-85% of GIST). A small proportion of GIST (~ 10-15%), defined as *KIT/PDGFRA* wild-type (WT), represents a group with distinct molecular hallmarks, including defects in succinate dehydrogenase (*SDH*) complex, and mutations of neurofibromatosis type 1 (*NF1*), *BRAF* or *KRAS* genes. Currently, little is known about differences in miRNA expression between *KIT/PDGFRA* mutant and *KIT/PDGFRA* WT-*SDH* deficient GIST. This prompted us to perform an integrated multiple expression profile of miRNA and mRNA, constructing an original miRNA-mRNA regulatory network in *KIT/PDGFRA* WT-*SDH deficient* GIST patients.

Patients and methods: Analysis were carried out on *KIT/PDGFRA* mutant versus *KIT/PDGFRA* WT-*SDH* deficient GIST. Genome-wide miRNA and gene expression analysis were performed using Agilent Human miRNA microarray and Affimetrix array, respectively .

Results: Three potential regulatory networks (*IGF1R* - miR-139-5p/miR-455/let-7b, *CDK6* - miR-139-5p/let-7b, *CD44* - miR-330-3p) were identified.

In summary, we performed a miRNA and mRNA analysis on KIT/PDGFRA mutant compared to *KIT/PDGFRA* WT-*SDH* deficient GIST, combining microarray analysis and bioinformatics integration through Ingenuity Pathway Analysis. We later confirmed with qRT-PCR that miR-139-5p, miR-330, miR-455-5p and let-7b were significantly up-regulated in *KIT/PDGFRA* mutant GIST compared to *KIT/PDGFRA* WT-*SDH* deficient GIST. In particular our results suggested that the miR-139-5p, 455-5p and let-7b signature may represent a potential onco-miR mark in *KIT/PDGFRA* WT-*SDH* deficient GIST, usually characterized by the *IGF1R* epigenetic enhanced expression, driving the carcinogenic development of *KIT/PDGFRA* WT-*SDH* deficient GIST. Indeed, in this context, epigenetically *IGF1R* activation and over-expression would serve the same as the driver *KIT/PDGFRA* mutation. Therefore, this miRNA signature has the potential to represent an important diagnostic tool and therapeutic targets in *KIT/PDGFRA* WT-*SDH* deficient GIST. In conclusion, we are aware of the limitation of the present study, in particular the small sample size that could have masked the significant effect of some of the miRNA analyzed, and consequently leading to the non-identification of important miRNA-mRNA networks. This means that, further identification of additional aberrantly expressed miRNA, in a larger GIST population, and the elucidation of their functional roles will be helpful in understanding the pathogenesis of GIST disease, and has the potential to represent a rational therapeutic strategy for the treatment of *KIT/PDGFRA* WT-*SDH* deficient GIST