Self-assembling nanoparticles for miRNA delivery towards precision medicine against melanoma

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In the last years two different therapies have significantly improved survival for patients with metastatic melanoma: immunotherapy and target therapy, which inhibits the mitogen-activated protein kinase (MAPK) pathway, almost always overactivated. However, only a subset of patients responds to these therapies against this highly aggressive tumor, which can be fatal within 18 months of diagnosis [1]. This limitation is due to drug resistance which can occur immediately or during therapy, after an initial positive response, compromising the long-term efficacy of target therapy. In recent years our group identified a set of microRNAs (miRNAs) responsible for the development of drug resistance to targeted therapy in BRAF-mutant melanoma. Some of these miRNAs are oncosuppressors and they were significantly downregulated in drug resistant tumors. We have demonstrated the capability of these miRNAs to prevent the development of drug resistance *in vitro* and *in vivo* [2-3]. However, the full exploitation of miRNAs and therapeutics requires the use of nanocarriers to prevent the rapid degradation of free nucleic acids in biological fluids, facilitating intracellular delivery [4]. These nanocarriers must be a versatile platform to facilitate the delivery to the point of interest, allowing a high cellular uptake and a long-term stability for a strong therapeutic efficiency. These challenges can be overcome through the use of self-assembling lipid nanoparticles (SANP) with a calcium phosphate core enclosed by a lipid shell [5]. SANPs are assembled before the use and enables the encapsulation of miRNAs tailored to the specific patient (SANP-miRNA), paving the way to personalized RNA-based therapies. Here, we present the generation of miRNA-loaded SANP, namely miR-199b-5p and miR-204-5p (briefly SANPs bis) in BRAF-mutant melanoma which showed high miRNA encapsulation efficiencies, good stability in serum and low hemolytic activity. SANP-miRNAs were able to induce a strong miRNA intracellular uptake, thereby impairing the proliferation of BRAF-mutated melanoma cell lines *per se* and better when combined with targeted therapies. Molecularly, we found that SANPs bis inhibit the release of soluble tumor-promoting factors such as VEGFA and TGFβ1, target genes of miR-199b-5p and miR-204-5p respectively. Finally, as further proof of concept of SANP platform versatility, we have tested whether SANPs delivering another oncosuppressive miRNA, i.e. miR-579-3p, were able to further enhance the antitumor ability of SANPs bis. miR-579-3p has been identified in our lab as an oncosuppressor because it targets the 3′UTRs of two oncoproteins, BRAF and MDM2 [6]. Preliminary results suggest that the combinations of SANPs bis and SANPs containing miR-579-3p alone with BRAF and MEK inhibiting drugs dramatically increased the percentage of tumor cells death. Altogether these results demonstrate the potential synergism and versatility of the SANP technology in combination with targeted therapy to prevent the development of drug resistance.

References:

[1] F. Valenti, *et al.* Precision Medicine and Melanoma: Multi-Omics Approaches to Monitoring the Immunotherapy Response. Int. J. Mol. Sci. 2021, 22.

[2] L. Fattore, *et al.* Reprogramming miRNAs global expression orchestrates development of drug resistance in BRAF mutated melanoma. Cell Death Differ. 2019, 26, 1267.

[3] L. Fattore, *et al.* Oncosuppressive miRNAs loaded in lipid nanoparticles potentiate targeted therapies in BRAF-mutant melanoma by inhibiting core escape pathways of resistance. Oncogene. 2023 Jan;42(4):293-307

[4] S. W. L. Lee, *et al.* MicroRNA delivery through nanoparticles. J. Control. Release 2019, 313, 80.

[5] V. Campani, *et al.* Hybrid lipid self-assembling nanoparticles for brain delivery of microRNA. Int. J. Pharm. 2020, 588, 119693.

[6] L. Fattore and R. Mancini, *et al.* miR-579-3p controls melanoma progression and resistance to target therapy. Proc Natl Acad Sci USA. 2016 Aug 23; 113(34): E5005–E5013.