Therapeutic *in vivo* RNAi-based nanoparticle silencing in lung tumor-associated macrophages

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Statement of Purpose: Excessive and prolonged activity of inflammatory macrophages associated with tumors is a hallmark of cancer progression. In fact, throughout tumor progression, tumor-associated macrophages (TAMs) overexpress angiogenic factors such as VEGF, which promotes cancer progression and metastasis. Hence, combined therapy against TAMs and VEGF can simultaneously repress cancer development [1]. Targeted siRNA therapy has been receiving increasing attention for down-regulating specific gene expression in cancer cells [2,3]. However, development of efficient delivery vehicles to immune cells has remained a major unmet challenge for turning siRNA into therapeutics [4,5]. We hypothesized that targeting nanoparticles for siRNA delivery could silence VEGF mRNA in the inflammatory tumor M2 macrophages aimed to selectively inhibit tumor progression in a lung cancer syngeneic orthotopic murine model. By targeting these specific immune cells we are inhibiting migration and consequently adverse function of these cells and their progeny. Rather than silencing a gene, we aim to eradicate completely this cell population from the inflammatory site.

Methods: Gold nanoparticles (AuNPs, ~15 nm) functionalized with thiolated-PEG-COOH conjugated to TAMs-targeting peptide (M2pep) and thiolated anti-VEGF siRNA labelled with Alexa Fluor 488 were produced. In vitro siRNA release and silencing efficiency was tested in stably transfected A549-luciferase-C8 human lung adenocarcinoma cells. Efficient and selective in vivo delivery of siRNA from functionalized AuNPs was tested in mice induced with lung cancer and treated with RNAi- and RNAi-M2pep NPs directly to bronchial airways (0.05 mg/kg of siRNA) for several time points.

Results: Highly potent and selective anti-vascular endothelial growth factor (VEGF) siRNA-M2pep AuNPs were administered via intratracheal instillation in mice, and are rapidly distributed in TAMs. Knockdown of VEGF in tumor-associated macrophages was confirmed at mRNA and protein levels, and RNAi-M2pep NPs were tested in a multi and long-term dosing system, using lung cancer murine model. Treatment with low doses of siRNA (ED50 0.0025-0.01 mg/kg) substantially reduced the recruitment of inflammatory TAMs in lung tumor tissue, reducing tumor size (~95%) and increased animal survival (~75%) in mice. These selective RNAi-M2pep NPs can induce specific, potent and long-lasting VEGF inhibition both in vitro and in vivo, specifically targeting inflammatory lung tumor-associated macrophages, via an immune modulation of the tumor milieu combined with tumor suppressor effects, with no signs of toxicity/inflammation.

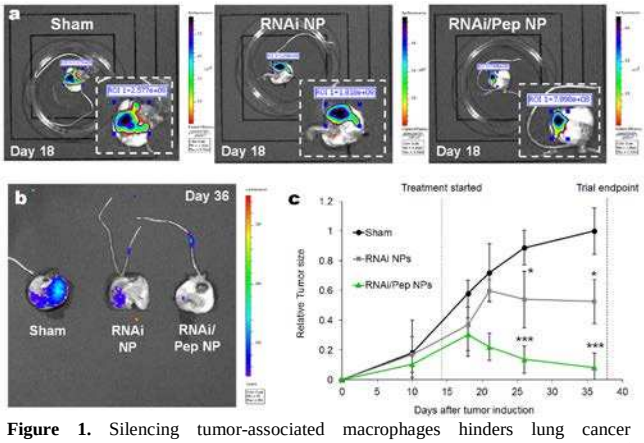

Figure 1. Silencing tumor-associated macrophages hinders lung cancer progression and enhances mice survival. (**a-b**) Bioluminescence analysis of luciferase-expressing A549-luciferase-C8 human lung adenocarcinoma cells in lungs from BALB/c mice, 18 (**a**) and 36 (**b**) days after treatment with RNAi- and RNAi-M2pep NPs, in a triple-dose (days 14, 18 and 21) of 0.05 mg/kg siRNA conjugated to NPs. (**c**) Relative tumor size in BALB/c mice bearing lung tumor xenografts treated with the RNAi- and RNAi-M2pep NPs, compared to untreated mice (sham). The mice were treated through repeated intratracheal instillation at 0.05 mg/kg of siRNA on days 14, 18 and 21. The tumor volume was monitored by luciferase bioluminescence (n = 6, * P < 0.05, *** P <0.005).

Conclusions: We developed a unique nanoformulation for molecular targeting of murine lung TAMs and demonstrated the potential of this approach as a viable, highly potent anticancer therapy. Our approach represents a paradigm shift from conventional silencing of specific oncogenes to combination therapy that eradicates an important cell population, TAMs, along with silencing angiogenic factor such as VEGF, which is critical for tumor progression. A similar targeted delivery strategy could be applied to treat other diseases associated with increased levels of specific markers expressed by resident cells in pathological conditions (other types of cancer, atherosclerosis, fibrosis and asthma).

References:

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