PROGRAMMA DI RICERCA STM

Il Fruitore: Valentina Russo

Istituto di afferenza: Dipartimento di Medicina molecolare e Biotecnologie mediche; Università di Napoli "Federico II"; IEOS-CNR; Naples, ITALY

con qualifica: PhD student

Istituto ospitante: Department of Pathology and Laboratory Medicine; Weill Cornell Medical College; New York, NY, USA

Periodo di permanenza: 10 luglio – 30 agosto 2015

In vivo anti-tumoral function study of an aptamer-miRNA conjugate.

Proponente:

Prof. Mario De Felice

Beneficiario:

Dr. Valentina Russo

Introduction

Lung cancer is the most frequently encountered tumor in the industrial world with increasing incidence in both men and women. It is also one of the most common malignant diseases and leading cause of cancer-related deaths worldwide with more than 200.000 new cases each year. Approximately 85% of all lung cancer cases are categorized as non-small cell lung cancer (NSCLC).

Recent studies have shown the importance of microRNAs (miRs) as key regulators in tumor initiation and progression, and also their great potential as new class of therapeutics cancer therapeutics. However, a major obstacle to their translation to clinic is actually represented by the lack of a robust and reliable way to selectively deliver them to the target malignant tumor cells.

We identified a tumor-suppressive miRNA in non-small cell lung cancer (NSCLC), miR-34c. We validated that the expression of miR-34c is low in NCSCL and when transfected into cell lines is able to impact on cell proliferation. (Fig. 1)

Nucleic-acid aptamers represent an expanding new class of biomolecules which is revealing as an interesting and highly promising for the specific delivery of RNA-based therapeutics.

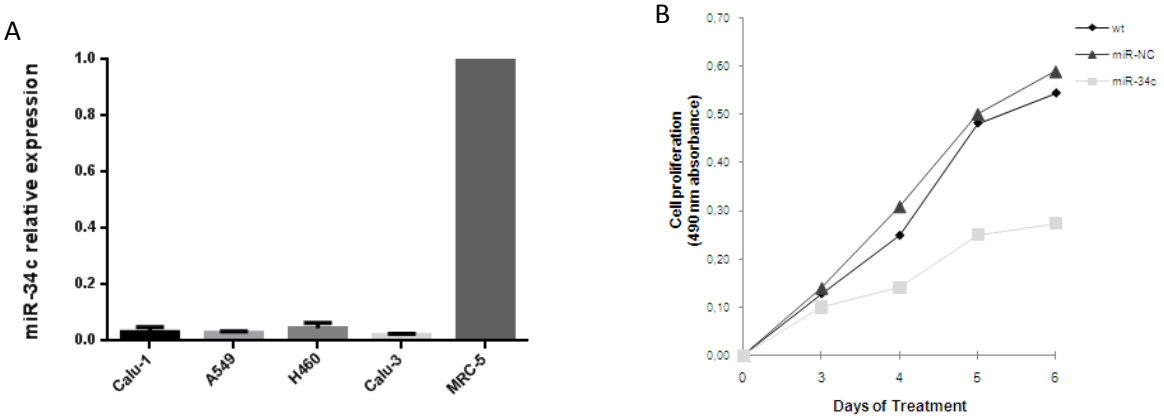

Fig. 1: A) The expression level of miR-34c in four NSCLC cell lines. B) cell proliferation in NSCLC cell line, untreated or transfected with either miR-NC or miR-34c.

Aim of project

In this study, we intend to validate the use of aptamers as cell-specific delivery molecules for “therapeutic” miRNAs, conjugating the miR-34c to a nucleic acid aptamer (GL21.T).

Results

Inghirami's lab has developed 3 patient derived tumor xenograft (PDTX) models from NSCLC patients. PDTXs are usually created implanting a fragment of a patient's primary tumor directly into an immunodeficient mouse, and, generally, they retain the histological characteristics of the patient parental tumor. Numerous studies showed that PDTX models preserve mutation profiles as well as the response patterns to targeted therapies.
For each PDTX mice model, primary cell lines have been created for in vitro experiments.

In preliminary in vitro experiments shown below.

Firstly, I evaluated AXL expression in tumors coming from three different patients, by qRT-PCR.
For each patient, I compared AXL expression in the primary tumor, in tissues and cell lines derived from three PDTXs, to A549 cells, NSCLC continuous cell, AXL+.
Two of the three patients (MB22 and PI23) had similar AXL levels to those of A549 cells.

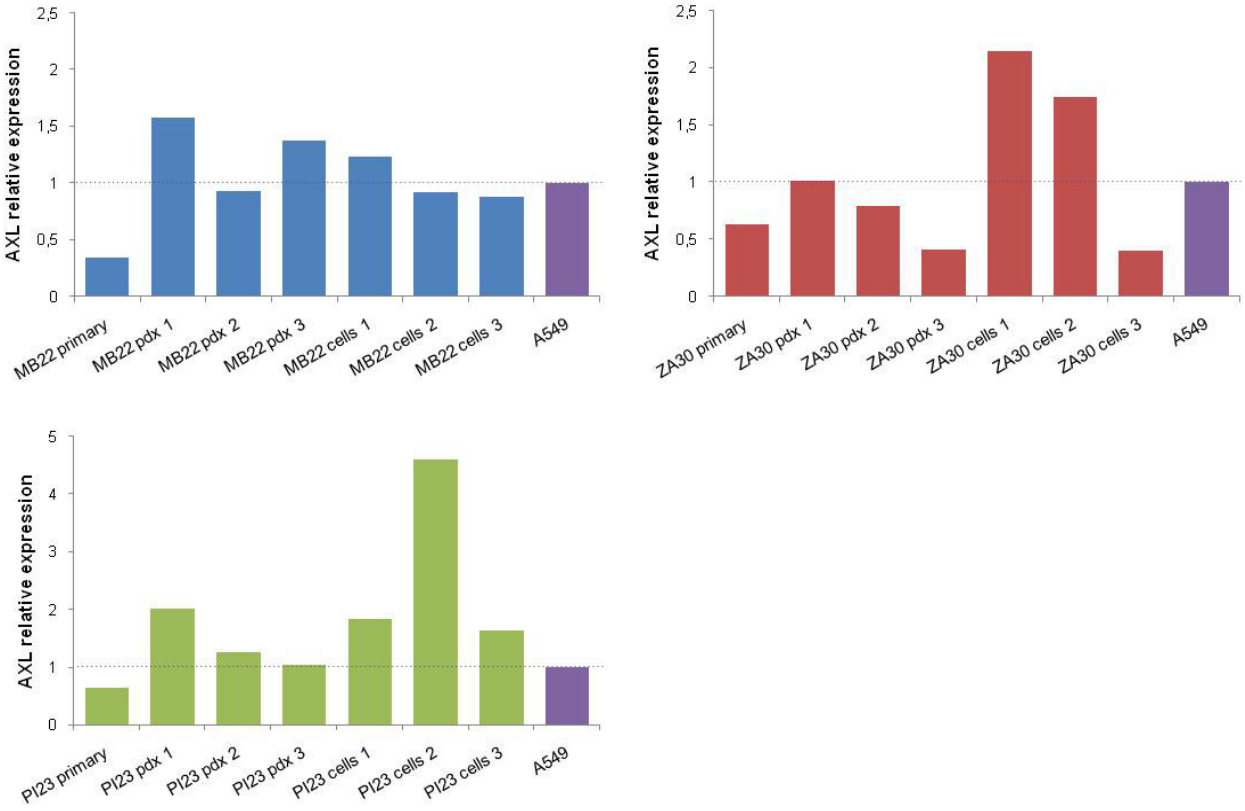

Fig. 1: AXL relative expression in 3 NSCLC patients.

Afterward, I performed a qRT-PCR analysis to detect the expression levels of miR-34c in the same patients. As shown in Figure 2, the endogenous levels of miR-34c in these samples were equivalent or lower compared to A549 cell line and, interestingly, the expression levels of miR-34c were lower in primary tumor compared to PDTX models (tissue and cells).

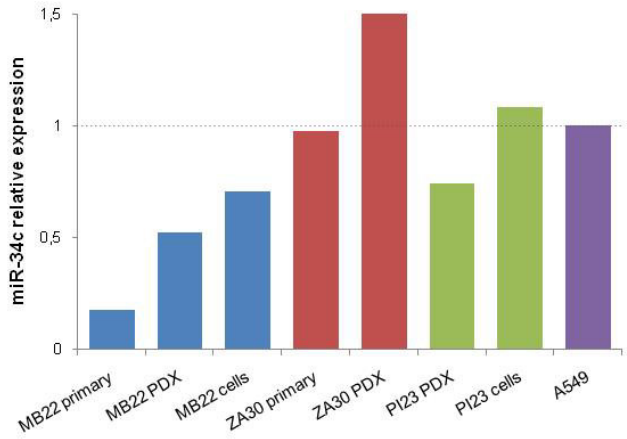

Fig. 2: miR-34c relative expression in 3 NSCLC patients.

Finally, I verified the correct annealing of GL21.T/34c and GL21.Tscra/34c conjugates by a non-denaturating gel electrophoresis analysis. In Figure 3, the annealed conjugates were clearly visible as a shift in band migration (lanes 4 and 6).

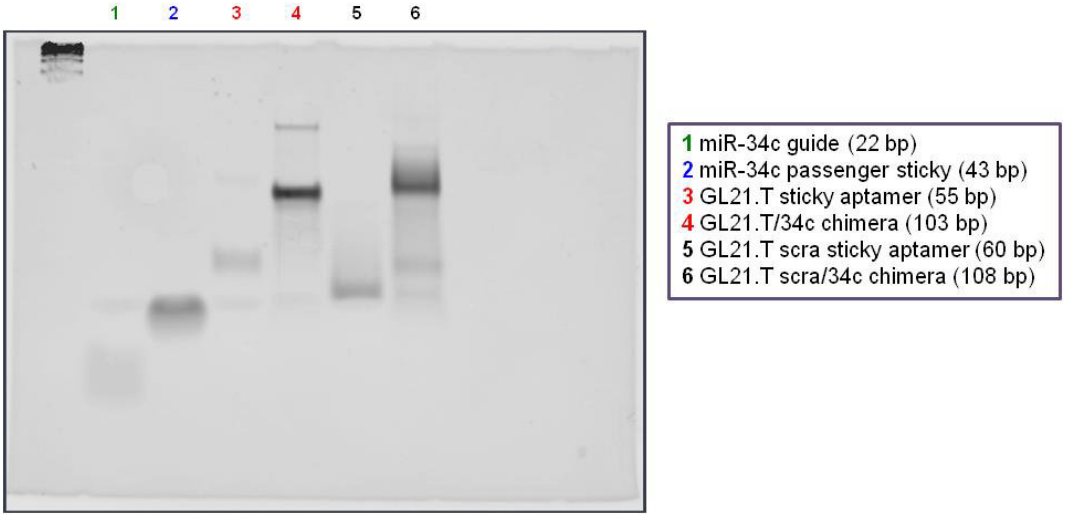

Fig. 3: RNA sequences and annealed conjugates were loaded on a 10% non denaturing polyacrilamide gel and stained with ethidium bromide.