SISTEMA SANITARIO REGIONALE
IRCCS
ISTITUTI FISIOTERAPICI
OSPITALIERI

ELENCO DOMANDE SPECIFICHE

Avviso pubblico, per titoli e colloquio, per l'assunzione a tempo determinato n. 1 risorsa nel profilo di ricercatore sanitario, categoria DS, per laureati in scienze biologiche, biotecnologie farmaceutiche o genetica e biologia molecolare - Prog. POC PI Prof. Gennaro Ciliberto
IFO Direzione Scientifica IRE - Via Elio Chianesi, 53 - Roma il 27/11/2023 alle ore 12:00

1. Parlare di evidenze scientifiche sul delivery di miRNA oncosoppressori da parte di nanoparticelle lipidiche (LNP) a cellule tumorali in vitro e in vivo

2. Parlare di possibili meccanismi di azione antitumorale che possono essere innescati nel melanoma da molecole di microRNA

Estratto il n. 1 domanda in evidenza

Istituti Fisioterapici Ospitalieri - ROMA

SISTEMA SANITARIO REGIONALE
IRCCS
ISTITUTI FISIOTERAPICI
OSPITALIERI

ELENCO DOMANDE INFORMATICA

Avviso pubblico, per titoli e colloquio, per l'assunzione a tempo determinato n. 1 risorsa nel profilo di ricercatore sanitario, categoria DS, per laureati in scienze biologiche, biotecnologie farmaceutiche o genetica e biologia molecolare - Prog. POC PI Prof. Gennaro Ciliberto
IFO Direzione Scientifica IRE - Via Elio Chianesi, 53 - Roma il 27/11/2023 alle ore 12:00

1. Cosa generalmente viene rappresentato in un grafico Volcano nell'analisi dei dati di espressione genica?

2. Cosa è un algoritmo?

Estratto il n. 1 domanda in evidenza

Istituti Fisioterapici Ospitalieri - ROMA

Blandino *et al. J Exp Clin Cancer Res* (2023) 42:189
https://doi.org/10.1186/s13046-023-02752-8

Journal of Experimental & Clinical Cancer Research

MEETING REPORT Open Access

The new world of RNA diagnostics and therapeutics

Giovanni Blandino[1*], Roberto Dinami[1], Marco Marcia[2], Eleni Anastasiadou[3], Brid M. Ryan[4], Alina Catalina Palcau[1], Luigi Fattore[5], Giulia Regazzo[1], Rosanna Sestito[6], Rossella Loria[7], Ana Belén Díaz Méndez[1], Maria Chiara Cappelletto[1], Claudio Pulito[1], Laura Monteonofrio[7], George A. Calin[8], Gabriella Sozzi[9], Jit Kong Cheong[10], Ranit Aharonov[11] and Gennaro Ciliberto[12]

Abstract

The 5th Workshop IRE on Translational Oncology was held in Rome (Italy) on 27–28 March at the IRCCS Regina Elena National Cancer Institute. This meeting entitled "The New World of RNA diagnostics and therapeutics" highlightes the significant progress in the RNA field made over the last years. Research moved from pure discovery towards the development of diagnostic biomarkers or RNA-base targeted therapies seeking validation in several clinical trials. Non-coding RNAs in particular have been the focus of this workshop due to their unique properties that make them attractive tools for the diagnosis and therapy of cancer.

This report collected the presentations of many scientists from different institutions that discussed recent oncology research providing an excellent overview and representative examples for each possible application of RNA as biomarker, for therapy or to increase the number of patients that can benefit from precision oncology treatment.

In particular, the meeting specifically emphasized two key features of RNA applications: RNA diagnostic (Blandino, Palcau, Sestito, Díaz Méndez, Cappelletto, Pulito, Monteonofrio, Calin, Sozzi, Cheong) and RNA therapeutics (Dinami, Marcia, Anastasiadou, Ryan, Fattore, Regazzo, Loria, Aharonov).

Keywords RNA structure, Cell cycle regulation, RNA evolution, Epigenetics, Splicing

*Correspondence:
Giovanni Blandino
giovanni.blandino@ifo.it
[1] Translational Oncology Research Unit, IRCCS, Regina Elena National Cancer Institute, Rome, Italy
[2] EMBL, Grenoble, France
[3] Department of Clinical and Molecular Medicine, Sapienza University, Rome, Italy
[4] MiRNA Therapeutics, London, UK
[5] SAFU Laboratory, IRCCS Regina Elena National Cancer Institute, Rome, Italy
[6] Preclinical models and new therapeutic agents Unit, IRCCS Regina Elena National Cancer Institute, Rome, Italy
[7] Unit of Cellular Networks and Molecular Therapeutic Targets, IRCCS Regina Elena National Cancer Institute, Rome, Italy
[8] MDAnderson, University of Texas, Houston, USA
[9] IRCCS, National Cancer Institute, Milan, Italy
[10] National University of Singapore Yong Loo Lin School of Medicine, NUS Centre for Cancer Research and Mirxes Lab Pte Ltd, Singapore, Singapore
[11] Pangaea Biomed, Tel Aviv, Israel
[12] Scientific Direction, IRCCS Regina Elena National Cancer Institute, Rome, Italy

© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Istituti Fisioterapici Ospitalieri - ROMA

Estratto il n. 1 domanda in evidenza

Exploring miRNAs/mRNAs network in breast cancer progression

Giovanni Blandino (IRCCS, Regina Elena National Cancer Institute, Rome, IT)

1

Metastasis is the leading cause of cancer related mortality. Metastasis is an evolutionarily dynamic and multifaced process along which tumor cells gain the ability to escape from the primary site and colonize distant organs. Unlike primary tumors, metastatic patients have not strikingly benefited from therapeutic advances. A major obstacle to designing precise treatment for metastatic disease relies on the lack of mechanistic details of the dissemination process. Growing evidence has reported that the mutational landscape of metastatic lesions resembles largely that of the originating primary tumors thereby limiting the use of precision drugs directed to specific druggable gene mutations. Altogether these findings suggest that epigenetic alterations and aberrant expression of RNA on both coding and non-coding components might endow unique information to decipher molecularly metastasis. To fulfill this specific aim, we combined bulk-RNA seq data with microRNA profiling of a given set of breast cancer mets to identify miRNA/mRNA networks with pro-metastatic activities (Fig. 1). To functionally assess the contribution of the identified network we are actively pursuing the generation of organoids derived from fresh breast cancer-derived metastatic tissue lesions (Fig. 1). We have recently shown that organoids derived from breast cancer metastatic lesions carrying PIK3CA mutations can be efficaciously treated with PI3K-a inhibitor, Alpelisib irrespective of the metastatic site [1, 2]. This unique 3D-preclinical platform has proven to mostly recapitulate the genomic alterations and gene expression patterns of the originating metastatic tissue; thereby providing a robust cancer surrogate to test the impact of miRNA/mRNA networks in the metastatization process and unveil new metastatic targets to be tacklen therapeutically.

Targeting TRF2 by LNPs-miR-182-3p in triple-negative breast cancer

Roberto Dinami (IRCCS, Regina Elena National Cancer Institute, Rome, IT). Telomeric repeat binding factor 2 (TRF2) is a member of the Shelterin complex involved in chromosome ends protection and telomere maintenance by T-loop formation [3, 4]. It is over-expressed in different cancer types and contributes to cancer progression [5, 6]. Here, we developed a miRNA-based approach to reduce TRF2 expression in vivo.

By performing a high-throughput luciferase screening, we identified miR-182-3p as a specific and efficient regulator of TRF2 able to induce DNA damage at telomeric and pericentromeric sites, eventually leading to strong apoptosis activation (Fig. 2A-D).

Interestingly, treatment with miR-182-3p-containing lipid nanoparticles (LNPs) induced tumor growth inhibition in advanced TNBC models including artificial brain

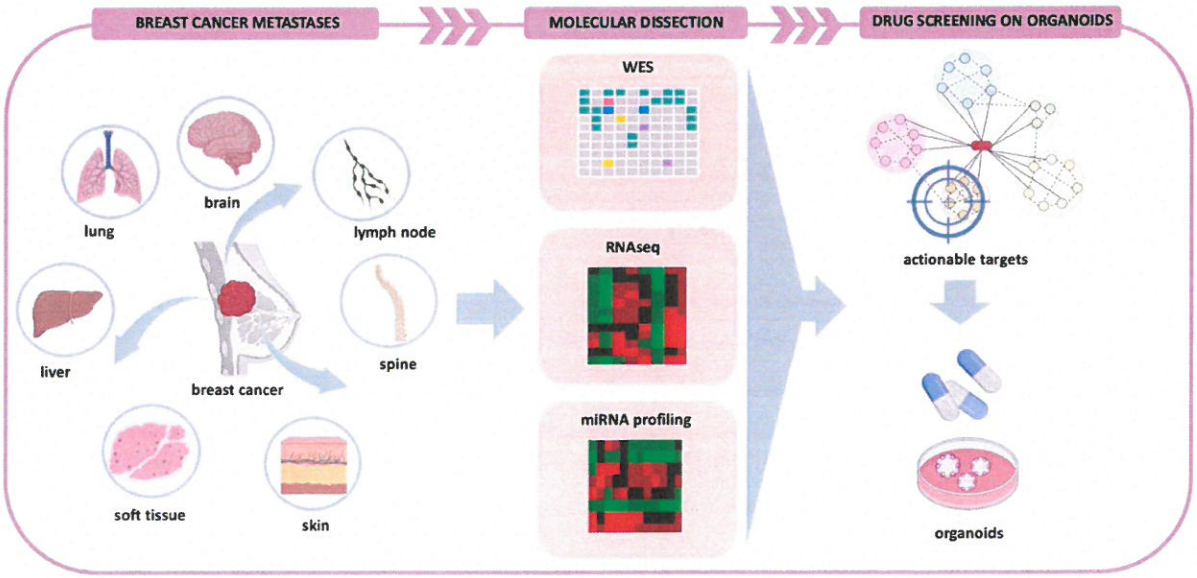

Fig. 1 Molecular dissection of breast cancer metastases. We collected breast cancer metastases from different sites, and we performed multiomic analyses, including whole exome sequencing (WES), bulk RNA sequencing (RNAseq), and miRNA profiling. The integrated analyses allowed to the identification of altered pathways in breast cancer metastases and novel therapeutic approaches that will be evaluated on patients-derived organoids

Istituti Fisioterapici Ospitalieri

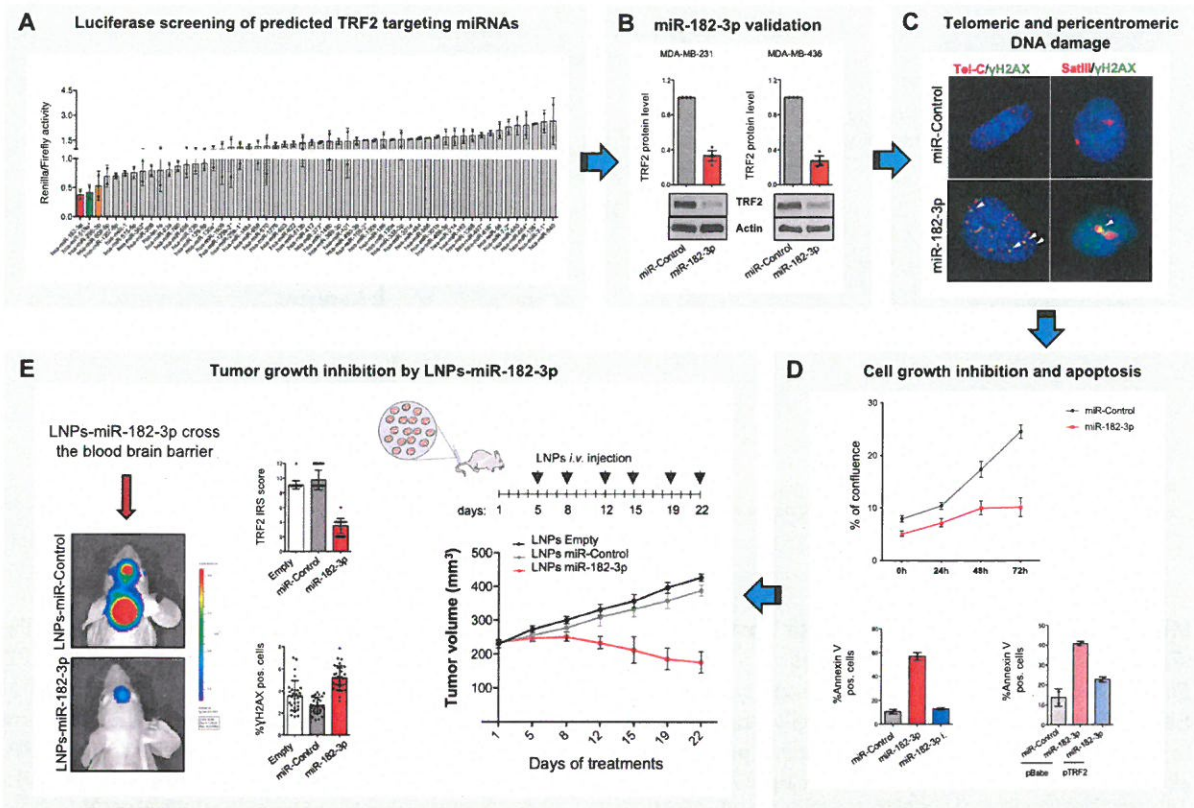

Fig. 2 Targeting TRF2 by LNPs-miR-182-3p in triple-negative breast cancer. **A** Targeting of 3'UTR-TRF2 by selected miRNAs was tested by high-throughput luciferase screening. **B** Western blot of TRF2 expression in tumor cells transfected with miR-182-3p or miR-Control is shown. **C** Representative images show the telomeric and pericentromeric DNA damage induced by miR-182-3p in MDA-MB-231 cells. **D** Cell confluence or apoptosis activation in MDA-MB-436 cells, transfected with the indicated miRNAs, were monitored by live-cell imaging (Incucyte) or analysed by flow cytometry (FACS), respectively. **E** Scheduling of treatment (top/right panel); Analysis of tumor inhibition after treatment with LNPs-miR-182-3p or its relative controls (bottom/right panel); TRF2 and γH2AX expression in tumor tissues was analyzed by immunohistochemistry (IHC) (middle panel); Representative images show mice with artificial brain tumor generated by intracranial injection of MDA-MB-231. Mice were treated with LNPs-miR-182-3p or its control (left panel)

metastasis (Fig. 2E). Our approach represents a possible therapeutic option for TNBC and its metastatic brain lesions.

Diversity and functional implications of long non-coding RNA structures

Marco Marcia (EMBL, Grenoble, FR). Long non-coding RNAs (lncRNAs) are key regulators of gene expression, playing active roles in epigenetics, transcriptional and translational regulation, and chromatin scaffolding. However, because of their recent discovery and molecular complexity, lncRNAs are still very poorly characterized from a mechanistic perspective raising outstanding biological questions on how they selectively and efficiently tune gene expression. Our work describes the mechanistic complexity of lncRNAs from an interdisciplinary evolutionary, cellular, and structural perspective. We have specifically dissected the structural and functional properties of a human alternatively spliced lncRNA, called MEG3 which stimulates the p53 pathway preventing tumorigenesis. This lncRNA adopts a well-defined structural core, dictated by tertiary interactions that are essential for p53 stimulation and for inducing cell cycle arrest. Remarkably, we could visualize its 3D structure for the first time by AFM and SAXS at ~ 15 Å resolution. More recently, we have identified specific protein regulators of our target lncRNA. Some of these proteins are p53 activators responsible for its basal p53-stimulatory activity, while others are p53 repressors that inhibit its tumor-suppressing function (Fig. 3). Our work connects the 3D structure and protein interacting network of lncRNAs to their biological function and opens still-unexplored research perspectives to understand lncRNA biology at high resolution for exploiting their translational potential in RNA-based therapies.

2

Istituti Fisioterapici Ospitalieri - ROMA -

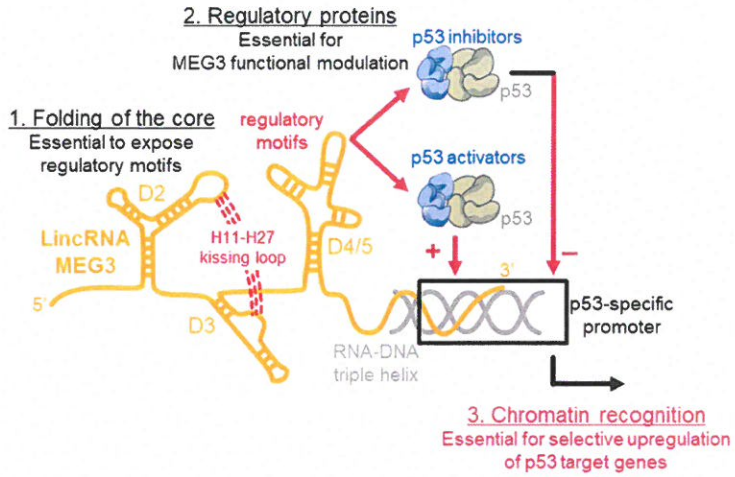

Fig. 3 Mechanistic hypothesis for the p53-stimulatory role of MEG3. Folding of the MEG3 core exposes regulatory motifs to p53 activators/repressors to recruit p53 on chromatin, and fine-tune the expression of p53 target genes

MiRNA-aided immunotherapies for Epstein-Barr virus (EBV)-associated lymphomas

Eleni Anastasiadou (Sapienza University, Rome, IT) EBV belongs to the herpesvirus family and contributes to more than 200.000 cases of cancers per year globally, including Burkitt lymphoma, nasopharyngeal carcinoma, gastric cancer, and diffuse large B-cell lymphoma. The virus escapes T cell recognition and exerts its oncogenic potential using its latency programs. In particular, the presentation focused on the molecular mechanisms underlying viral immune escape through its major transforming protein, namely, Epstein-Barr nuclear antigen 2 (EBNA2). This viral protein induces the programmed death ligand-1 (PD-L1) immune checkpoint (IC) expression in DLBCL cell lines by downregulating PD-L1 targeting miR-34a [7]. We have recently shown how EBNA2 recruits early B-cell transcription factor 1 (EBF1) on the miR-34a promoter to silence its expression. Further, by using 3D microfluidic models, [7] we demonstrated for the first time that overexpression of miR-34a promotes T cell infiltration and killing of EBNA2 expressing DLBCL cells. These results paved the way for an international patent on RNA-aided immunotherapies (No.: WO2019232160). Using this model system, it was further shown that, the combination of miR-34a and anti-PD-L1 antibodies was more efficient in increasing the tumor immunogenicity than either anti-PDL1 or miR-34a alone. Subsequently, we illustrated how the same EBV-encoded protein negatively affects the inducible T cell costimulator (ICOSL). EBNA2 reduces ICOSL by upregulating miR-24 (unpublished data). Thus, EBNA2 seems to compromise tumor immunogenicity by simultaneously increasing PD-L1 by downregulating miR-34a and reducing ICOSL expression by increasing miR-24 (Fig. 4). Since cancer immunotherapies based on only anti-PD-L1 antibodies have shown low overall response rate (ORR), we propose a novel combinatorial miRNA-aided immunotherapy approach in which, IC targeting miRNAs in combination with antibodies may prove useful and provide a better outcome of immunotherapies [8].

Applications of RNA activation in oncology

Bríd M. Ryan (MiRNA Therapeutics, London, UK). Small activating RNAs (saRNAs) are short double-stranded oligonucleotides that activate transcription within the nucleus through an evolutionarily conserved mechanism. MiNA Therapeutics has successfully designed saRNAs against a wide range of drug targets, including transcription factors, cytokines, receptors, and intracellular proteins. The translational read across of saRNA, therefore, extends from haematology to autoimmune diseases, metabolic diseases, and oncology, among others. MTL-CEBPA is a first-in-class saRNA therapy [9, 10]. Its mechanism of action involves upregulation of the transcription factor C/EBP-α (CCAAT/enhancer-binding protein alpha), which is a master regulator of the myeloid cell lineage. Decreased C/EBP-α expression frequently occurs in the context of cancer, with loss of C/EBP-α leading to a block in myeloid differentiation and an accumulation of MDSCs [11]. The saRNA is packaged within myeloid-targeting lipid nanoparticles and, when delivered to myeloid cells, restores C/EBP-α protein to normal levels to drive differentiation of immature myeloid cells towards a less immunosuppressive phenotype. Overcoming immunosuppression in the TME is a key step in the cancer immunity cycle (CIC). MDSCs are linked with both primary

...oterapici Ospitalieri - ROMA