



UNIVERSITY
OF TRENTO - Italy



Laboratory of RNA Biology and
Biotechnology

The RNA strategy

RNA as a tool and target in human disease diagnosis and therapy.

The Laboratory of RNA Biology and Biotechnology at the Centre for Integrative Biology (CIBIO) of the University of Trento, Italy, is focusing on RNA to develop better diagnostic tools and therapeutics for a range of genetic diseases and cancers. The research projects developed in the laboratory also aim at unveiling new active roles for RNA molecules in the mechanisms underlying these diseases.

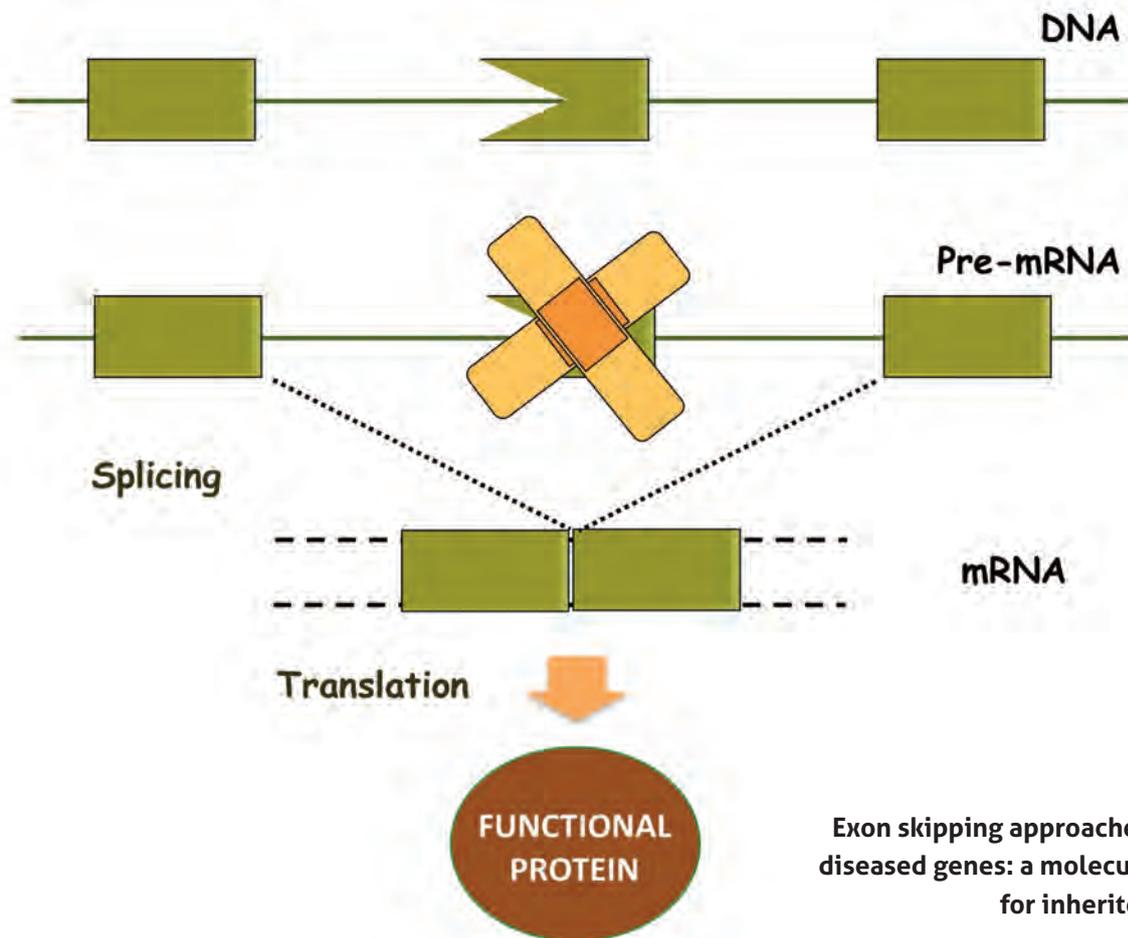
RNA impacts nearly every aspect of gene expression and it is now clear that a large portion of human genetic diseases are caused by mistakes in RNA metabolism. It has become progressively evident that RNA is not just a carrier of genetic information, but also a catalyst and a guide for sequence-specific recognition and processing of other RNA molecules.

The growing body of knowledge concerning RNAs is opening up exciting and unprecedented avenues for research: RNA molecules are today, at the same time, targets of therapeutic intervention, tools for functional studies and novel therapeutic molecules to treat human diseases¹.

Led by Dr Michela Alessandra Denti, the research in the RNA Biology and Biotechnology laboratory covers various diverse areas, and researchers focus on two main research interests.

¹ Denti MA, Viero G, Provenzani A, Quattrone A, Macchi P (2013) mRNA fate: life and death of the mRNA in the cytoplasm. RNA Biol. 10:360-366.





Modulating RNA splicing as a cure for inherited diseases

The laboratory studies different ways of modulating 'RNA splicing', a process that all messenger RNAs (mRNAs) undergo in the cell. Several gene mutations, which cause different rare inherited diseases, affect the splicing of specific mRNAs. At variance with mainstream gene therapy approaches, which aim at replacing the mutated gene with a functional DNA copy of the gene itself, Denti and coworkers intend to correct the mRNA transcribed from the mutated gene, through an approach called "exon-skipping"^(2,3). By introducing small RNA molecules, they mask the mRNA to the attack of the splicing machinery, inducing it to jump certain portions of the mRNA, thus restoring the correct message. The team predicts that exon-skipping holds a great deal of promise as a

potential cure for genetic diseases, and several clinical trials have supported the notion that the technique could one day become commonplace.

The group is carrying out studies to develop a cure for a rare neurodegenerative disease: Frontotemporal Dementia with Parkinsonism linked to chromosome 17 (FTDP-17). FTDP-17 patients have single nucleotide mutations in the gene for protein *tau*, which induce the aberrant inclusion of an exon in *tau* mRNA, and ultimately cause the accumulation of this protein in neurons and consequently result in neuron's degeneration. Very recently, Denti and colleagues have been successful in inducing exon skipping and the correction of *tau* mRNA

through the use small RNA molecules in cells. They are now working to show the efficacy of the approach in an animal model of the disease.

They are also exploring the possibility to apply exon skipping to other genetic disorders, such as retinal dystrophies and metabolic diseases.

2 Siva K, Covello G, Denti MA (2014) "Exon-skipping antisense oligonucleotides to correct mis-splicing in neurogenetic diseases" *Nucleic Acids Therapeutics*. 24:69-86. doi:10.1089/nat.2013.0461.

3 Bacchi N, Casarosa S, Denti MA (2014) "Splicing-correcting therapeutic approaches for retinal dystrophies: where endogenous gene regulation and specificity matter." *Invest Ophthalmol Visual Sci*. in press

MicroRNAs as biomarkers of cancer, cardiac and neurodegenerative diseases

A second line of research is aimed at studying some very small RNA molecules called microRNAs (miRNAs) that have only recently been discovered. Due to their size these RNA molecules were overlooked for a long time, but it has become clear in the last decade that thousands of them are encoded in the genomes of all organisms, and play a crucial role in cells, fine-tuning the production of proteins. The deregulation of miRNAs has been implicated in several diseases, and the laboratory is studying the potential for specific miRNA, to be used as a tool in the early and accurate diagnosis of cancers, cardiac diseases and neurodegenerative diseases.

Biomarkers able to stratify the subtype of cancer, prognosticate the course of disease, or predict the response to treatment are in increasing demand. A perfect biomarker should have some important features: non-invasivity, specificity,

early detection, sensitivity and ease of translatability from model systems to humans.

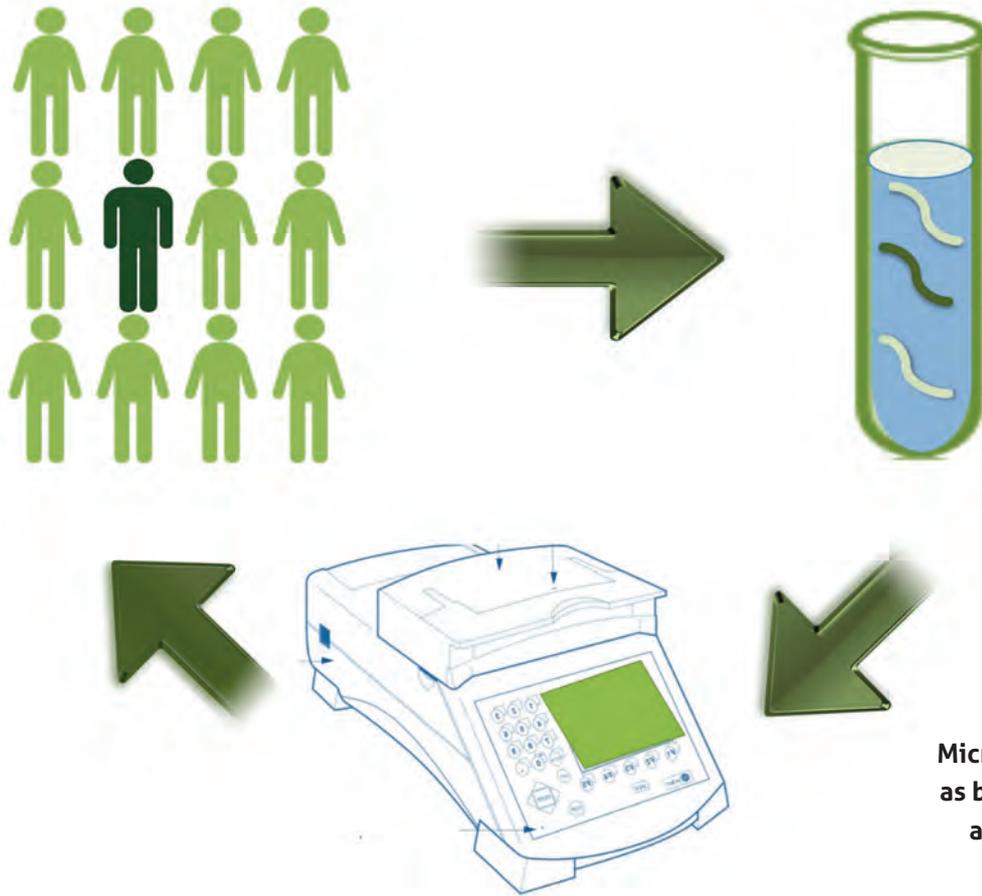
Indeed in the last ten years, alteration of miRNA levels and/or correlation of miRNA expression with clinical parameters (such as disease progression or therapy response), have been shown in several cancers, indicating that miRNAs can serve as clinically relevant biomarkers.

The advantage of the use of miRNAs as biomarkers resides in the ease of their detection and in their extreme specificity. Furthermore, miRNAs are remarkably stable molecules that have been shown to be well preserved in formalin fixed, paraffin embedded bioptic tissues as well as in fresh snap-frozen specimens, unlike larger RNA molecules as mRNA. These tiny molecules are also preserved, detectable and quantifiable in the circulation and in other biofluids, such as urine, saliva, cerebro-spinal

fluid and amniotic fluid, and their concentration can reflect altered physiological conditions, representing new effective biomarkers.

The tiny size of these RNA molecules, originally contributing to their obscurity, turned out to be an advantage, when it came to their stability and the specificity of their detection.

In one project, the group focused on lung cancer, the leading cause of cancer mortality worldwide ⁴. As it is now possible to design therapies targeted towards specific forms of cancer, Denti and her colleagues were required to distinguish between squamous cell carcinomas (SCCs) and adenocarcinomas (ADCs). To do this, the researchers measured the quantities of miR-205 and miR-21 in cancer biopsies, and found that the results provided a useful marker for differentiating between SCCs and ADCs – an identification



MicroRNAs have been proposed as biomarkers for the diagnosis and prognosis of cancers and several other diseases.

which is not always possible via traditional histochemical methods^(5,6). Recently, the researchers have discovered that measuring other miRNAs could enable the differentiation between neuroendocrine tumours both from the other two lung tumour types, and amongst themselves.

In other projects, the group is actively pursuing the goal of identifying a miRNA signature from bioptic samples or plasma, that could aid in the diagnosis and prognosis of cardiac diseases or frontotemporal dementia.

The group is also working towards the identification of molecular mechanisms in which miRNAs play a key role, thus representing optimal targets for a therapeutic approach^(7,8).

4 Del Vescovo V, Grasso M, Barbareschi M, Denti MA (2014) "microRNAs as lung cancer biomarkers." *World Journal Clinical Oncology*. in press

5 Barbareschi M, Cantaloni C, Del Vescovo V, Cavazza A, Monica V, Carella R, Rossi G, Morelli L, Cucino A, Silvestri M, Tirone G, Pelosi G, Graziano P, Papotti M, Dalla Palma P, Doglioni G, Denti MA (2011) "Heterogeneity of large cell carcinoma of the lung: an immunophenotypic and miRNA based analysis." *Am. J. Clin. Pathol.* 136:773-782.

6 Del Vescovo V, Cantaloni C, Cucino A, Girlando S, Silvestri M, Bragantini E, Fasanella S, Cuorvo LV, Dalla Palma P, Rossi G, Papotti M, Pelosi G, Graziano P, Cavazza A, Denti MA, Barbareschi M (2011) miR-205 expression levels in non-small cell lung cancer do not always distinguish adenocarcinomas from squamous cell carcinomas." *Am. J. Surg. Pathol.* 35:268-275

7 Bisio A, De Sanctis V, Del Vescovo V, Denti MA, Jegga AG, Inga A, Ciribilli Y (2013) Identification of new p53 target microRNAs by bioinformatics and functional analysis. *BMC Cancer* 13:552 doi:10.1186/1471-2407-13-552

8 Del Vescovo V, Meier T, Inga A, Denti MA,

Borlak J (2013) A Cross-Platform Comparison of Affymetrix and Agilent Microarrays Reveals Discordant miRNA Expression in Lung Tumors of c-Raf Transgenic Mice. *PLoS ONE* 8(11): e78870. doi:10.1371/journal.pone.0078870



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