Identification of mutant p53-associated non-coding RNA network

22

Silvia Di Agostino, Giulia Fontemaggi, Fabio Valenti, Andrea Sacconi, Sara Donzelli, Magdalena Pruszko, Matteo Pallocca, Claudio Pulito, Elisa Milano, Federica Ganci, Sabrina Strano, Giovanni Blandino

Oncogenomic and Epigenetic Unit, Department of Research, Diagnosis and Innovative Technologies, IRCCS Regina Elena National Cancer Institute, Rome Italy

Background: Somatic missense mutations in *TP53* gene occur in over half of all human cancers and may impact the residues involved in direct contact with DNA (DNA contact mutants) or substitute the amino acids required for proper p53 protein folding and structure (conformational mutants). Mutations in the p53 protein may not only disrupt its wild-type tumor suppressing function but also confer new oncogenic properties (GOF, gain-of-function). Long noncoding RNAs (lncRNAs) belong to a class of ncRNAs that are longer than 200 nucleotides. Several studies have shown that lncRNAs may act as important cis- or trans-regulators in various biological processes. Mutations in lncRNAs or deregulation of their expression are associated with a wide range of diseases, especially cancers and neurodegenerative diseases through diverse and poorly understood molecular mechanisms.

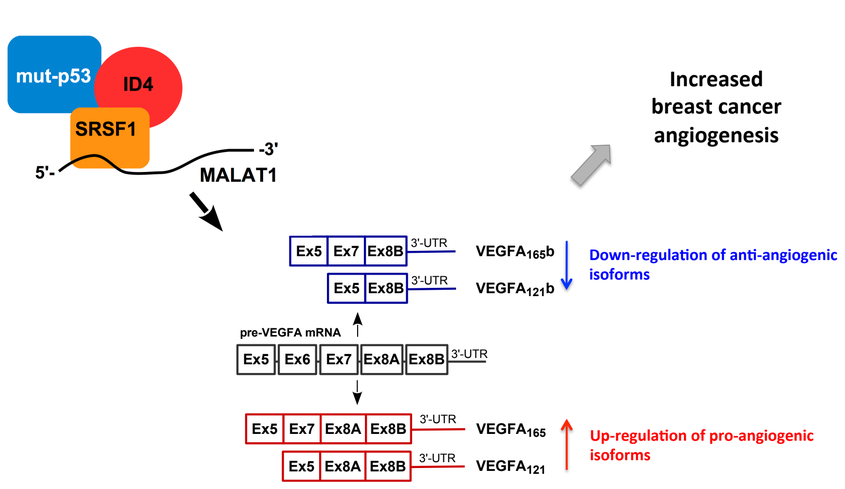
Aims: Our studies aim at deciphering mechanisms of functional interaction between mutant p53 protein and lncRNAs, mainly in head and neck squamous cell carcinoma (HNSCC) and in basal-like breast cancer (BLBC), both characterized by a high incidence of *TP53* oncogenic mutations.

Results: We characterized the role of specific tumor-derived mutant p53 proteins in the aberrant transcription of lncMIR205HG gene in HNSCC. We observed that MIR205HG was transcriptionally regulated by mutant p53 in CAL27 and FaDu HNSCC cell lines. We validated the association between *TP53* mutations and high MIR205HG expression in the TCGA (The Cancer Genome Atlas) HNSCC cohort and in a cohort of HNSCC patients from our Institute (IRE cohort). Down-regulation of MIR205HG expression significantly reduced cell proliferation, cell migration and clonogenic activity of HNSCC cancer cells. Mechanistically, MIR205HG depleted endogenous miR-590-3p leading to increased cyclin B, cdk1, and YAP protein expression. Taken together, these findings identify a transcriptional and post-transcriptional molecular network that includes mutp53 protein, lncMIR205HG, YAP, and other proliferation-related genes, which are enriched in HNSCC patients with poor prognosis.

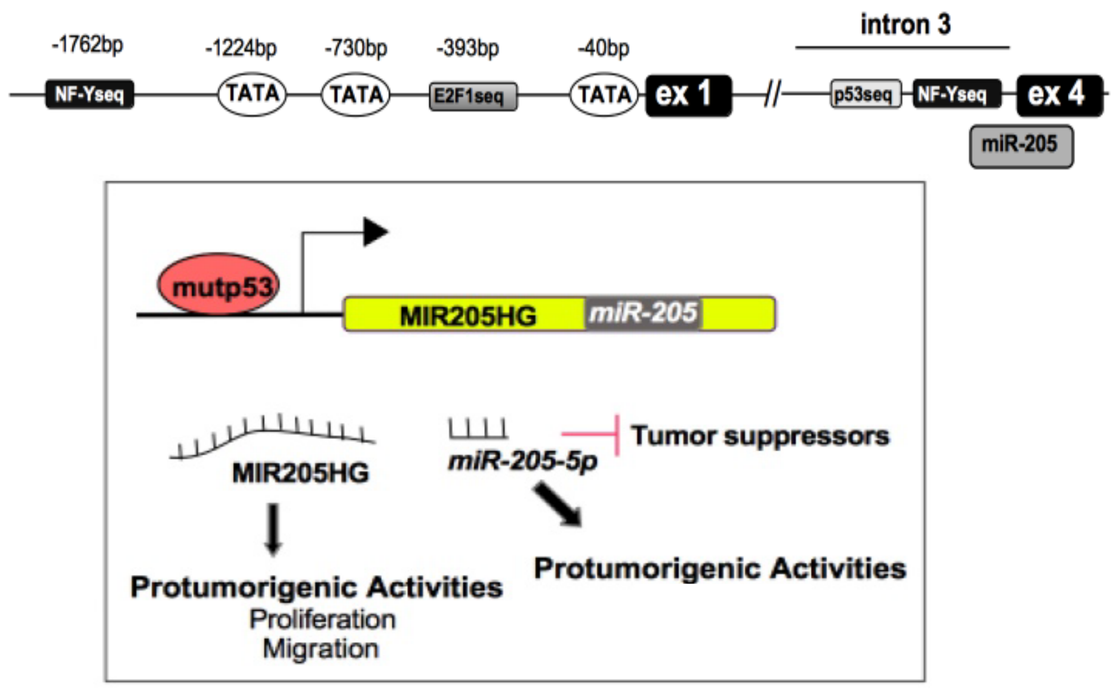
A second lncRNA that we have identified as component of the mutant p53-associated network is Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1). This lncRNA interacts with a subset of splicing factors and modulates their activity. In our study, we demonstrated that oncogenic splicing factor SRSF1 bridges MALAT1 to mutant p53 and ID4 proteins in breast cancer cells. Mutp53 and ID4 delocalize MALAT1 from nuclear speckles and favor its association with chromatin. This enables aberrant recruitment of MALAT1 on VEGFA pre-mRNA and modulation of VEGFA isoforms expression. Interestingly, VEGFA-dependent expression signatures associate with ID4 expression specifically in basal-like breast cancers carrying *TP53* mutations. Our results highlight a key role for MALAT1 in control of VEGFA isoforms expression in breast cancer cells expressing gain-of-function mutant p53 and ID4 proteins.

Conclusions: We assessed that mutant p53 proteins are able to both control the expression of lncRNAs and regulate their oncogenic activity. We believe that the deciphering of the *TP53* mutation-associated network of non-coding RNAs, including lncRNAs and miRNAs, might provide powerful targets for the design of novel therapeutic approaches aimed at counteracting mutant p53 gain of function and therapy resistance.

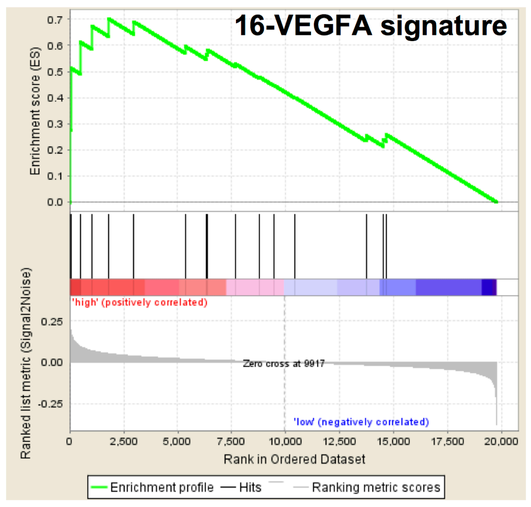
The mutant p53-ID4 complex controls VEGFA isoforms by recruiting lncRNA MALAT1



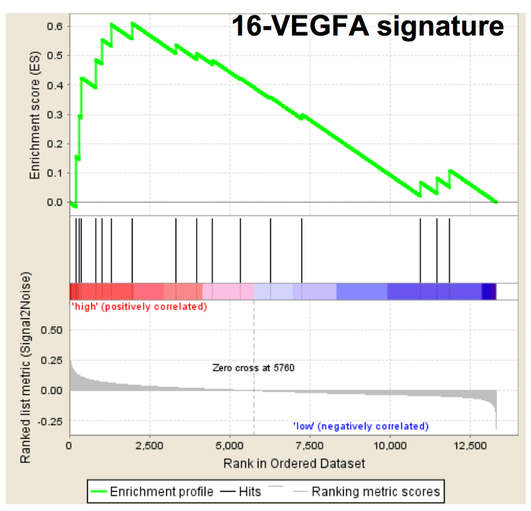
NF-Y and E2F1 mediate recruitment of mutant p53 to the MIR205HG promoter and upregulate the expression of lncMIR205HG and miR-205-5p in HNSCC



VEGFA signature expression associates with ID4 expression in mutant p53-carrying basal-like breast cancers

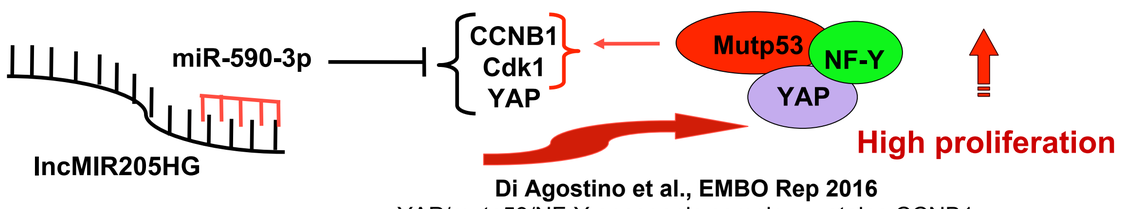


Metabric cohort, mutp53 BLBC ID4-high vs ID4-low



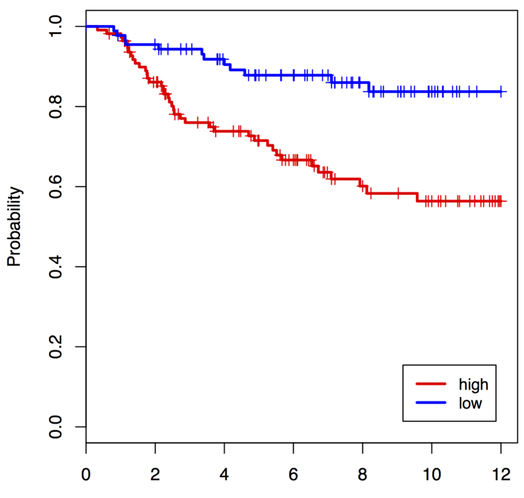
Compendium cohort, BLBC ID4-high vs ID4-low

LncMIR205HG acts as a natural decoy for miR-590-3p, leading to elevated expression of Cyclin B, Cdk1 and YAP proteins

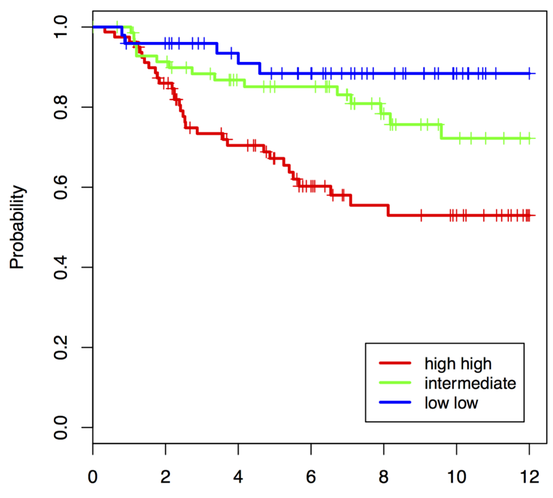


Di Agostino et al., EMBO Rep 2016
YAP/mutp53/NF-Y oncogenic complex sustains CCNB1 and CDK1 mRNAs transcription

16-VEGFA core signature expression

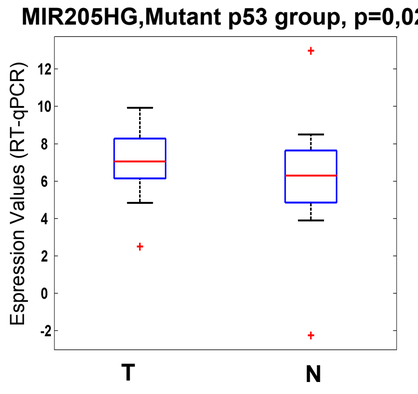


Combined ID4/16-VEGFA core signatures expression

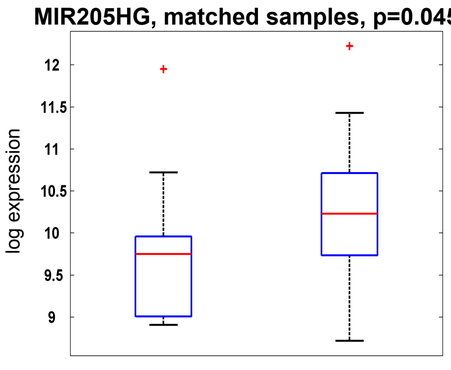


Pruszko M et al., EMBO Rep. 2017;
doi: 10.15252/embr.201643370

TP53 mutations are associated with high expression of lncMIR205HG in HNSCC patients



IRE cohort, 32 matched samples



TCGA_HNSCC

Di Agostino et al., Theranostics 2018
doi: 10.7150/thno.22167.

IRE ISTITUTO NAZIONALE TUMORI REGINA ELENA

15th Nov 2018

ISAB