IDENTIFICATION OF GENOMIC AND PROTEOMIC SIGNATURES IN LYMPHOMAS BY INDUCIBLE shRNA

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ABSTRACT

Anaplastic Large Cell Lymphomas (ALCL) are characterized by chromosome translocations in which the Anaplastic Lymphoma Kinase (ALK) gene is fused to several partners, most commonly the NPM gene. We have previously demonstrated that the constitutive activation of ALK tyrosine kinase induces cellular transformation and lymphomas. However, the molecular mechanisms leading to NPM-ALK mediated transformation and its requirements for tumor maintenance are still unclear.

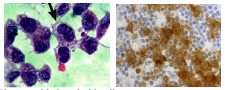
Towards this end, we targeted ALK expression in ALCL cells by lentiviral-mediated inducible small hairpin RNA (shRNA) and applied a comprehensive approach to investigate the resulting gene expression profile and signaling protein modulations. Specific NPM-ALK knock down determines modulation of known downstream effectors, followed by growth arrest and apoptosis. These effects corresponded to growth inhibition and tumor regression in a human *in vivo* xenograft model.

By means of gene expression profile analysis we determined the signature of NPM-ALK modulated genes in ALCL cells and identified known and novel downstream targets grouped in the functional cluster of cell cycle and proliferation, adhesion and migration, and in cytokine signaling.

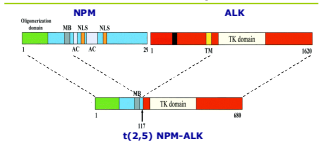
A proteomic analysis of immunoprecipitated protein samples from parental and NPM-ALK-knocked down cells allowed us the identification of new NPM-ALK substrates and interactors.

In conclusion, the combination of RNA interference with gene expression profiling and proteomic analysis represent a powerful tool to characterize the signaling network mediating tumorigenesis and maintenance of ALK positive lymphomas and to individuate suitable targets for a therapeutic intervention.

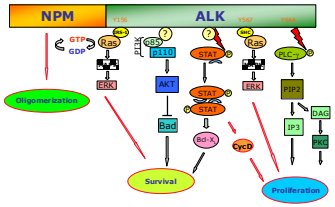
Pathology of ALCL



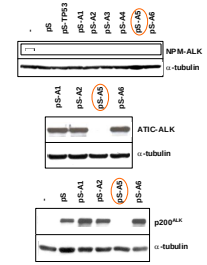
NPM-ALK fusion protein



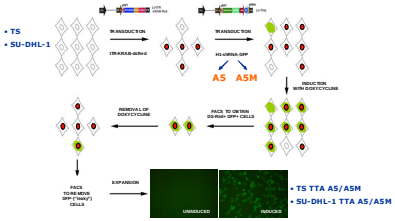
Oncogenic signaling cascade activated by NPM-ALK



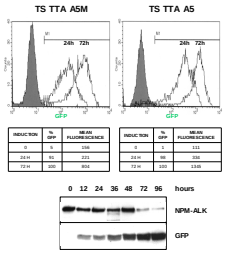
Selection of anti-ALK shRNA



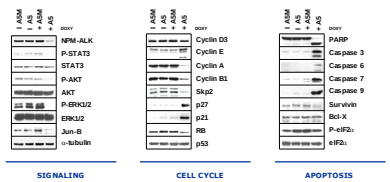
Generation of shRNA-inducible ALCL cell lines



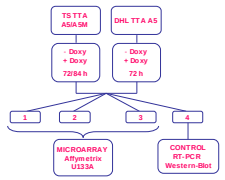
Tight silencing of NPM-ALK by inducible RNAi



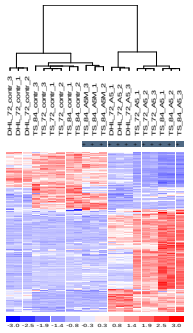
NPM-ALK silencing induces cell cycle arrest and apoptosis in ALCL cells



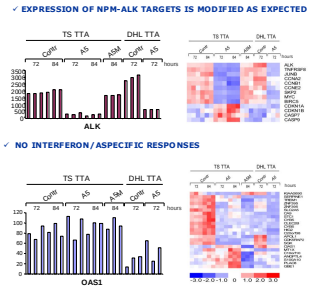
Microarray experiment



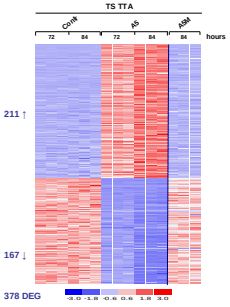
Unsupervised clustering



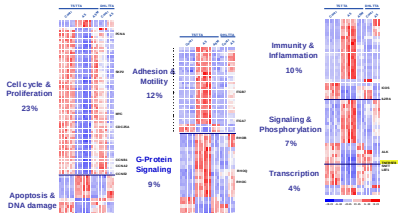
The specificity of the NPM-ALK silencing approach



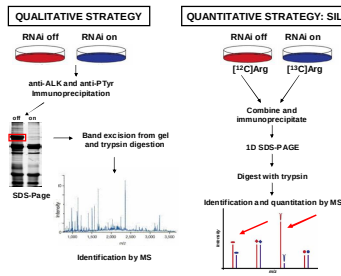
The NPM-ALK signature in the ALCL cell line TS



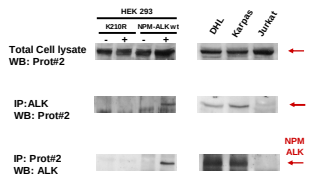
Distribution of the NPM-ALK signature into functional categories



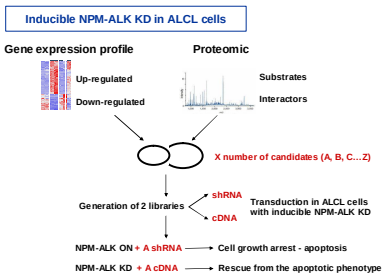
Proteomic: experimental strategies



CROSS-IMMUNOPRECIPITATIONS TO VALIDATE THE PROTEIN IDENTIFICATION AND INTERACTION



Biological validation of new NPM-ALK putative targets



CONCLUSIONS

We demonstrated that ALCL cells are dependent on ALK expression for both proliferation and survival.

We provided evidences that the use of inducible RNA interference in lymphoma research is a powerful tool to:

1. validate molecular targets for therapeutic intervention;

2. discover new oncogenic signals by gene expression profile and by proteomic approaches.