MODELING ROS1 TRANSLOCATION IN LUNG CANCER: A TRANSLATIONAL MODEL TO TEST DRUG'S EFFICACY

1)Pizzino G. 2)Kannan R. 3)Altavilla D. 4)Squadrito F. 5)Ventura A. 6)Bitto A.

University of Messina

Cancers are characterized by numerous somatic mutations, of which only a subset contributes to the tumor's progression. These "driver" mutations have to be distinguished from the pre-ponderance of neutral "passenger" mutations. Chromosomal rearrangements disrupting the open reading frame of oncosuppressor genes (thus causing their inactivation), or producing fu-sion genes by chromosome translocations, drive tumorigenesis. Sequencing approaches con-firmed that numerous, non-clonal translocations are a typical feature of cancer cells, driving ~20% of cancer cases. These rearrangements contain DNA sequence from multiple genomic sites, produced via non-homologous end joining (NHEJ).

ROS Proto-Oncogene 1 Receptor Tyrosine Kinase (ROS1) is an orphan receptor that encodes for a type I integral membrane protein with tyrosine kinase activity and activates several downstream signaling pathways related to cell differentiation, proliferation, growth and survival. Ros1 is vulnerable to chromosomal rearrangements, resulting in transforming gene fusions typical of several cancers, the most representative of which is the non-small cell lung cancer (NSCLC). So far, there is a lack of animal models mimicking chromosomal rearrangements in-volving Ros1, but several Ros1 fusion partners genes, were identified sequencing human NSCLC samples. The break points of Ros1 are exons 32, 34 and 35. All of the break points allow the resulting fusion to harbor the kinase domain of ROS1. The mechanism by which ROS1 fusion proteins become constitutively active is currently unknown. For other cancer related RTK fusions, such as ALK, the fusion partner provides a dimerization domain that induces con-stitutive oligomerization and thus activation of the kinase. However, for ROS1, it is unclear whether dimerization is involved in activation of both the wild-type and the mutated receptor.

To produce a validated mouse model we used the CRISPR system that uses non-coding RNAs to guide the Cas9 nuclease to induce site-specific DNA cleavage. These damages will be repaired by cellular DNA repair mechanisms, via NHEJ or homology directed repair (HDR).

We first performed in vitro experiments to model the Lrig3-Ros1 translocation in NIH3T3 murine fibroblasts; we cloned the two sgRNAs targeting Lrig3 and Ros1 respectively into a Cas9-expressing plasmid and co-transfected the resulting constructs in mouse cells, achieving the translocation. By sequencing, we verified that the fusion transcript matched perfectly the one reported in literature. The sgRNAs designed allowed us to edit mouse genome, produce the Lrig3-Ros1 translocation, and generate a stable mouse cell line carrying this mutation, useful to determine the molecular mechanism by which it act as pro-oncogenic factor.

After this, we built an AAV vector expressing Cas9 and the sgRNAs targeting Lrig3 and Ros1 (AAV-LR); this vector can be used to infect the lung epithelium of adult mice, to test the in vivo oncogenic potential of Lrig3-Ros1 translocation.

We infected C57/Bl6 wild-type mice with our AAV-LR vector, to check if Lrig3-Ros1 translocation was able to induce lung cancer by itself.

We infected p53-/- mice as well, to assess if mice lacking of p53 were more suitable to model the translocation we were interested in.

Moreover, we decided to assess if the same Lrig3-Ros1 translocation can lead to cancer in a context of Telomerase reverse transcriptase (TERT) over-activation, a common mechanism supporting, cancer onset and progression.

To re-activate TERT in adult mice lungs, we employed a more advanced dCas9-based SAM sys-tem, developed by engineering the single gRNA (sgRNA) through appending a minimal hairpin aptamer to the tetraloop and stem loop 2 of sgRNA. Such an aptamer was able to enhance the recruitment of transcription factors around the target gene promoter and thus facilitate the po-tency of dCas9-mediated gene activation.

We co-infected 12-weeks old CAST/EiJ mice a strain characterized by having telomeres almost same length of the homo sapiens ones) with a dCas9 SAM Adeno-vector designed to induce TERT re-activation and Adeno-Lrig3-Ros1, in order to assess the effects of this genomic lesion combined to TERT over-expression.

We have been able to model Lrig3-Ros1 dependent lung cancer in both p53-/- mice, and CAST/EiJ TERT-overactive mice; we also verified that it is responsive to crizotinib (100mg/kg per os, once a day for 14 days). We are now working to better characterize the tumor samples, both histologically and molecularly, and to replicate these results in a larger cohort of animals.