Modeling human cancer mutations *in vivo*: CRISPR/Cas9 impact in cancer biology and pharmacology

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In cancer cells, only a small subset of somatic mutations contribute to the tumor's progression. These 'driver' mutations have to be distinguished from the preponderance of neutral 'passenger' mutations.
The dramatic increase in sequencing data from human cancer samples raised the need of assessing whether or not each identified mutation is a driver or a passenger one.
This is a key step in terms of cancer biology, letting us to better understand how do cancer initiates, how do it progress, why different cancers are more or less aggressive, and how do it acquires the ability to spread in whole body and give raise to secondary metastatic cancers. Nonetheless, to achieve the long-time aimed precision medicine approach we can not ignore the need of animal models able to mimic human cancer diseases with high fidelity in terms of genetic alterations; this in order to acquire a pre-clinical pharmacological platform to test new targeted anti-cancer drugs, as well as to assess the pharmacological profile, in terms of efficacy, of the already available ones when comes to treat cancers with different genetic backgrounds.
CRISPR/Cas9 is a recently discovered genome editing tool which allow us to induce a broad spectrum of genetic alterations; by using this system we can produce insertions/deletions, inversions, translocations, and point mutations. Also, the system allow to activate or silence one or more-than-one genes simultaneously. It is important to note that all of these applications can be performed both in vitro and in vivo.
ROS Proto-Oncogene 1 Receptor Tyrosine Kinase (ROS1) is an orphan receptor tyrosine kinase (RTK), encoded by Ros1 gene, that activates downstream signaling pathways related to cell differentiation, proliferation, growth, and survival. The mechanism by which ROS1 fusion proteins become constitutively active is currently unknown. It is also unknown if this recently identified genomic rearrangements acts as driver in NSCLC.
Telomerase reverse transcriptase (TERT) is responsible for catalyzing the addition of nucleotides in a TTAGGG sequence to the ends of a chromosome's telomeres, preventing degradation of the chromosomal ends following multiple rounds of replication.
About 90% of cancers shows an increase in TERT activity; of all of them, lung cancer is the most well characterized TERT-associated tumor.
To understand if Lrig3-Ros1 translocation can be the driver mutation in lung cancer, we modeled the Lrig3-Ros1 translocation in NIH-3T3 cell line, then we packaged the CRISPR components into an AAV vector, infected mice via intra-tracheal instillation, and monitored them via uCT to detect the presence of a tumor mass.
Beside this, we applied the Synergistic Activator Mediator (SAM) CRISPR system targeted to activate TERT gene, in order to model TERT over-expression first in vitro then in vivo, infecting mice lung the same way we described previously.