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Investigations of Personalized Medicine in Mesothelioma

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INVESTIGATION OF PERSONALIZED MEDICINE IN MESOTHELIOMA

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ABSRACT

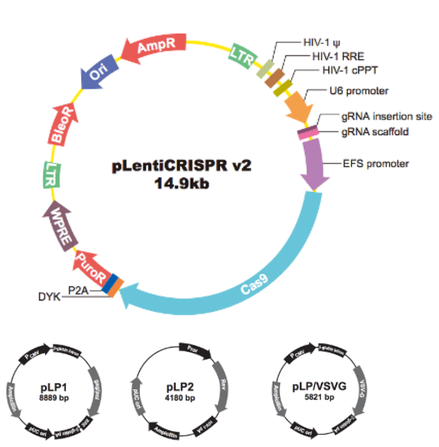
Malignant mesothelioma is a neoplasm that involves lesions on the pleural linings of the lung or the peritoneal lining of the abdominal cavity. Current standard of care involves a multi-targeted antifolate drug Pemetrexid and Cisplatin. This regimen results in regression of tumors in 25% of patients. As the genetics lesions that cause mesothelioma have been elucidated, our understanding of possible targets of therapeutics has grown. Rational drug design would dictate that we consider genetic alterations specific to mesothelioma tumor cells and use those to target our therapy. The most common genetic alterations in human mesotheliomas are the loss of function of three tumor suppressor genes, PTEN, NF2, and CDKN2A. In this project, we propose to induce hemizygous genomic deletions of the PTEN locus by using CRISPR/Cas9 nuclease-generating lentiviral vectors targeting PTEN in a “normal” mesothelial cell model, LP9/htert. Once established as a model system with various genomic alterations, the model system will be used to test a small set of front line chemotherapeutic agents for any increased therapeutic index. Theoretically, genes and proteins down stream of these genetic lesions will make some cancer cells more susceptible to specific inhibitors. Among the drugs to be tested are novel PI3 Kinase and mTOR inhibitors.

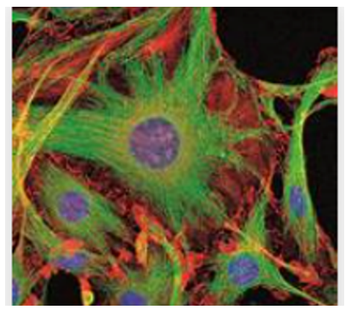
HYPOTHESIS

Our hypothesis is that PTEN knockout by CRISPR/Cas9 editing will result in an increase in sensitivity of suppressed cells to novel inhibitors directed at the pathways affected.

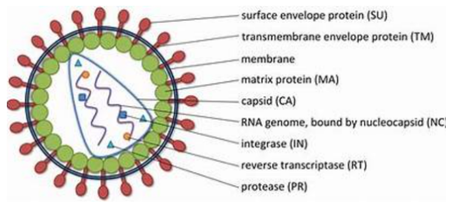
METHODOLOGY

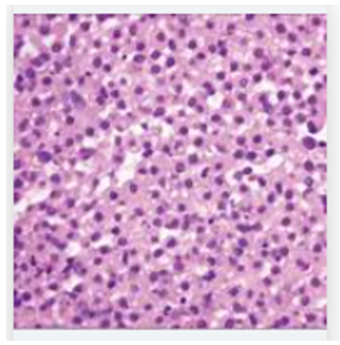
- CRISPR/Cas9 containing vectors were purchased from GenScript. (Piscataway, NJ)
- These vectors were co-transfected with virus packaging vectors to produce infectious viral particles that were used to transduce LP9/hTert "normal" mesothelial cells.
- Puromycin-resistant clones were selected, sequenced and stored for later analysis.
- Appropriate clones will be assayed for mRNA expression of the targeted gene, PTEN.
- Appropriate clones will be assayed for the level and size of protein (PTEN).
- The ability of PTEN to allow a robust apoptosis in the presence of interventions (UV light, camptothesins) will be assessed.
- The sensitivity of knockout clones to novel chemotherapeutic agents will be assessed compared to parental LP9/hTert cells.

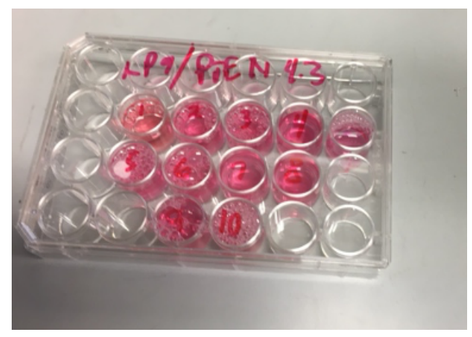




HEK293FTcells







RESULTS

We were able to create CRISPR/Cas9 knockouts of LP9 mesothelial cells targeting PTEN, as well as document retention of the CRISPR/Cas9 vector by Puromycin resistance in the resultant clones. The alteration of the sequence at the target exon (exon 3), of PTEN was documented. This alteration appeared to be hemizygous, as predicted in the literature (homozygous PTEN knockouts are senescent). Lastly, primers have been designed for qPCR analysis of PTEN mRNA expression.

RELATED LITERATURE

This research was approved by the Institutional Biosafety Committee (IBC); IBC 2022-001, IBC 2021-021, and IBC 2016-03