Generating and characterizing novel prostate cancer cell lines that employ the Alternative Lengthening of Telomeres (ALT) telomere maintenance mechanism

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Background: A key hallmark of cancer is unlimited replication, which requires cancer cells to evade replicative senescence induced by telomere shortening. The majority of cancers overcome this critical barrier by up-regulating the enzyme telomerase, a telomere-specific reverse transcriptase. However, for a subset of cancers that lack telomerase, telomeres are maintained by employing the Alternative Lengthening of Telomeres (ALT) pathway, which is thought to be dependent on homologous recombination. In a variety of tumor types, our laboratory and others have reported a robust correlation between ALT and recurrent cancer-associated somatic inactivating mutations in *ATRX* or *DAXX*, genes encoding chromatin-remodeling proteins. In a previous comprehensive survey of ALT in cancer, we reported that ALT was highly prevalent in some tumor types (e.g. astrocytoma, sarcomas, pancreatic neuroendocrine tumors), but we did not observe any ALT-positive prostate cancer cases. Notably, however, we found a subset of metastatic prostate cancer harbors ALT, suggesting that mutations giving rise to ALT in this disease are unique to metastatic prostate cancer.
Methods: Here, we have created the first prostate cancer cell lines exhibiting ALT, with the purpose of molecularly characterizing ALT in prostate cancer and identifying promising therapeutic approaches for men with lethal metastatic prostate cancer harboring this phenotype. Inactivating mutations in either *ATRX* or D*AXX* using the CRISPR cas9 nickase system were introduced into genetically well-characterized prostate cancer cell lines, LAPC-4 and CWR22Rv1.
Results: In this new model, abolishing ATRX expression was sufficient to induce the ALT phenotype in both LAPC-4 and CWR22Rv1, as assessed by multiple biomarkers of ALT, including the presence of bright telomeric FISH foci, ALT-associated PML bodies (ABPs), and c-circles. Interestingly, abolishing DAXX expression induced ALT in only a subset (3/5) of LAPC-4 KO clones.
Conclusions: Ultimately, we plan to use these isogenic cell lines to further characterize and elucidate the basic biology of cancers harboring ALT, and pharmacologically target the molecular features unique to the ALT phenotype. The identification of ALT-specific drugs may pave the way for the development of new targeted treatments for a subset of men with lethal metastatic prostate cancer that harbor this unique molecular phenotype, and more broadly, other cancers that share the ALT phenotype in common.

Conflicts of Interest: None

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