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Initial Cellular Characterization and Therapeutic Targeting of the Alternative Lengthening of Telomeres Phenotype in PNETs: Role of Telomeric Replication Stress

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BACKGROUND

A small subset of cancers (~10%) utilize a telomerase-independent telomere maintenance mechanism termed, Alternative Lengthening of Telomeres (ALT), which is present in ~40% of PNETs. Clinical studies have shown that ALT positivity in PNET patients correlates with both worse prognosis and clinical outcome. ALT+ cells have heightened levels of telomeric replication stress (stalling of the replication fork), which is believed to act as both a trigger and catalyst for ALT activity. ALT+ cancers are reliant on sustained replication stress-induced telomere damage to promote telomere elongation. Yet, replication stress and telomere damage levels must be kept within strict thresholds, and a fine balance must be struck to maintain cellular viability in ALT+ cancers. Importantly, relatively little is known regarding ALT in PNETs. With replication stress-induced DNA damage and its subsequent repair playing important roles in actively driving ALT, we are focused on selectively targeting key players and pathways pertinent to funneling the cause and effect of replication stress through the ALT phenotype in PNETs, for therapeutic benefit.

METHODS

Our overarching research goal is to selectively increase replication stress levels in ALT+ PNETs past a viable threshold. It was previously shown in ALT+ sarcoma cells that loss of FANCM, a DNA repair protein that limits replication stress, results in a “hyper-ALT” phenotype leading to massive replication stress-dependent cell death. As proof of concept for targeting ALT in PNETs, targeting FANCM, in our hands, involved inducible shRNA-mediated high-level knockdown of FANCM in ALT- (QGP-1) and ALT+ (NT18-P) PNET cell lines using a Dox-inducible lentiviral shRNA system. Cell viability was determined through clonogenic assays. Western blot analysis was performed to confirm FANCM knockdown and to quantify potential changes in replication stress levels.

RESULTS

Clonogenic assay results confirmed that targeting of FANCM, using our Dox-inducible lentiviral shRNA system, selectively decreased cell viability in the ALT+ PNET cell line, NT18-P. Western blot analysis confirmed high-level knockdown of FANCM in both PNET cell lines, with significantly higher levels of replication stress markers found in the ALT+ cell line.

CONCLUSIONS

ALT+ PNET cells are selectively sensitive to FANCM loss, with corresponding higher levels of replication stress and reduced cell viability. Ongoing studies are currently focused on targeting regulators of the ALT phenotype and replication stress management using small molecule inhibitors. With limited treatments for patients with advanced PNETs available, specifically targeting the ALT phenotype may offer innovative treatment regimens for nearly half of all PNET patients.

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