**MECHANISM-BASED COMBINATION THERAPY IN CANCER: STUDIES ON CANCER CELLS**

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**BACKGROUND AND OBJECTIVE**

Telomeres are DNA repeats at the ends of chromosomes that protect genome integrity along with specialized proteins that bind telomeres. Telomeres shorten with every cell division and a specialized enzyme telomerase can counteract it by adding telomeric repeats. In fact, telomerase activity, silenced in terminally differentiated somatic cells, is reactivated in cancers and there is a striking correlation between telomerase activity and cancer progression [1]. Because cancer cells have shorter telomeres than normal cells, exploring telomere-targeted therapeutics has the promise of treating not just one type but most types of cancer [2]. The objective of our study was to characterise a potential telomere-targeting drug, to identify combinatorial strategies that would sensitize cancer cells to the drug, and lastly, to uncover the underlying mechanism of action.

**METHODS**

We used established human cancer cell lines as model of study, to rapidly test promise of the drug and to find effective combination treatments. The cell types were KNS60, A172 (glioblastoma multiforme) and ONS76 (medulloblastoma). The telomere-targeting drug in study was TMPyP4, a porphyrin, that recognises and binds G-quadruplex forming regions with high specificity. The combination strategy we used was γ-radiation (4 Gy). We assessed telomerase activity using the Telomeric Repeat Amplification Protocol; levels of telomerase and telomere-associated proteins using western blotting. We measured DNA damage by single cell gel electrophoresis (Comet assay) and chromosome and telomere aberrations using Peptide-Nucleic Acid FISH of metaphase chromosome spreads. We used MTT assay and Cell Titer Glo to assess cell viability.

**RESULTS AND DISCUSSION**

At an LC50 dose of 100 µM for 48 hours, TMPyP4 inhibited telomerase activity significantly in the cancer cells accompanied by a significant reduction in the levels of the catalytic protein constituting telomerase, hTERT. In addition, TMPyP4 also reduced the level of c-MYC, an important oncogene, and a master transcriptional regulator. C-MYC is known to have a G-quadruplex forming region in its promoter, so TMPyP4 could be binding there and blocking its transcription. TMPyP4 also caused significant DNA damage, read out by damaged DNA tails via electrophoresis, and activation of DNA repair sensors like ATM kinase.

While it was clear that TMPyP4 mounted a comprehensive anti-cancer effect, we also tested TMPyP4 with a low dose of 1 – 10 µM and chronic treatment up to 8 weeks by passaging cells regularly. Remarkably, TMPyP4 at these low doses shortened telomeres progressively and caused telomeric and chromosomal aberrations in the cancer cells.

**Figure 1** DNA damage induction (tail moment, left panel) measured by comet assay and relative cell viability (right panel) in cells treated with TMPy4 and γ-radiation.

Given the promise of TMPyP4 to disrupt telomere maintenance in cancer cells, we sought to identify how effective it would be in combination with gamma-radiation that induces DNA damage (measured as tail moment in Figure 1). Indeed, in combination with 4 Gy radiation, TMPyP4 was effective even at half its LC50 dose as evidenced by relative viability (Figure 1). The combination treatment induced much greater DNA damage than either one alone. Because TMPyP4 causes DNA damage – both directly and indirectly via disrupting telomere maintenance, we think that radiation acts as a great combination strategy.

**CONCLUSIONS**

TMPyP4 effectively had a profound effect on cancer cells both at the molecular level as well as at the cellular level. TMPyP4 was not only potent with an acute dose over a short term, but also over a therapeutically relevant long-term treatment window with chronic low doses in the cancer cell types. TMPyP4 induced telomere shortening and chromosome aberrations in those cancer cells over time thus disrupting telomere maintenance. While TMPyP4 inhibited telomere maintenance, the inhibition of either DNA-PKcs or ATM kinase led to an exacerbated effect on DNA damage, cell arrest and cell viability of the cancer cell types tested. The intricate crosstalk of telomere maintenance and DNA repair factors could underlie this effect. Exploring this combination in a context closer to in vivo cancer models will prove as a promising hunt towards new standard of care of clinically recalcitrant cancers.

**REFERENCES**

[**1**] SHAY J.W., WRIGHT, W.E.. Mechanism-based combination telomerase inhibition therapy. Cancer Cell. 7 (2005), 1-2. [**2**] BARTHEL, F.P., WEI, W., TANG, M., et al. et al. Systematic analysis of telomere length and somatic alterations in 31 cancer types. Nat Genet. 49 (2017),349-357.