Alternative Lengthening of Telomeres Renders Cancer Cells Hypersensitive to ATR Inhibitors

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Modern conceptual models of cancer growth and development describe 6 critical biological capabilities of tumor cells: 1) self-sustaining growth signaling; 2) evading growth suppressors; 3) resisting cell death; 4) enabling replicative immortality; 5) inducing angiogenesis; and 6) activating invasion and metastasis. Each of these hallmark capabilities, in turn, is a key target for therapeutic intervention.

Telomeres play a central role in the continuous duplication of proliferating cells, until the erosion of the telomere eventually leads to cell mortality. Cancer cells overcome this fate in one of two ways: 1) through activating telomerase, an enzyme that extends telomere length; or 2) through activating alternative mechanisms, mostly likely involving genetic (homologous) recombination. Approximately 10-15% of human cancers utilize telomerase-independent mechanisms to maintain limitless replication potential. In particular, the ALT pathway is activated in approximately 5% of cancers, although the prevalence of ALT use varies substantially by tumor type. For instance, up to 60% of osteosarcomas and 40-60% of glioblastomas rely on ALT to escape replicative mortality.

Telomerase utilizes RNA-directed DNA synthesis to extend telomeres. In contrast, the ALT pathway involves recombination with telomeric DNA sequences and replication of telomere DNA. However, single-stranded DNA (ssDNA) coated by replication protein A (RPA) is a key intermediate in both DNA replication and homologous recombination. Telomeres are transiently associated with RPA during DNA replication, but RPA is released from telomeres after S phase. The association of RPA with telomeres in S phase is facilitated by a type of RNA called TERRA (telomeric repeat-containing RNA), which is also present at telomeres during this phase of the cell cycle.

To gain a better understanding of ALT-mediated telomere elongation, Dr. Zou and colleagues at the Massachusetts General Hospital Cancer Center recently mapped a model of critical steps along the ALT pathway. First, researchers examined variations in the cell-cycle regulation of TERRA. In telomerase-positive cells, TERRA levels fluctuate during the normal cell cycle as RPA binds to and detaches from RNA. By comparison, some ALT-positive cell lines do not exhibit normal fluctuations in TERRA throughout the cell cycle, perhaps due to the absence of active telomerase. Instead, these cells rely on the ALT pathway to lengthen telomeres through recombination with telomeric DNA sequences from the same or other chromosomes. The transcriptional regulator ATRX is commonly deleted or abnormally expressed in cells that utilize ALT, and the loss of ATRX leads to dysregulation of TERRA. In ALT-positive cells, ATRX loss led to the persistent association of RPA with telomeres after DNA replication, creating a recombinogenic nucleoprotein structure.

Whether the ALT pathway can be exploited therapeutically remains unknown. Another line of analysis assessed whether ATR is required for ALT functionality. Tests across multiple cell types treated with the ATR inhibitor VE-821 showed that ATR inhibition leads to:

- Increased telomere loss in ALT cells
- Increased double-strand breaks in ALT cells
- Induction of higher levels of genomic instability in ALT cells than in non-ALT cells
- Induction of higher levels of DNA damage in ALT cells than in non-ALT cells

By disrupting ALT and triggering chromosome fragmentation, ATR inhibition is associated with apoptosis in ALT cells. Importantly, the cell death induced by ATR inhibitors is highly selective for ALT cells across a panel of cancer cell lines. For instance, ALT-positive SW26ALT cells were more sensitive to VE-821 than telomerase-positive SW39Tel cells. In addition, at a concentration that killed ALT-positive USO2 osteosarcoma cells, VE-821 only moderately slowed the proliferation of untransformed RPE-1 retinal pigment epithelial cells.
**Summary**

ATR inhibition is a promising strategy for the treatment of ALT-positive cancers. Cancers that are reliant on recombination to elongate telomeres—including but not limited to ALT-positive cancers—demonstrate particularly sensitivity to ATR inhibitors. Several investigational ATR inhibitors are currently entering clinical trials for the treatment of multiple tumor types.

**Financial Disclosures**

Dr. Zou discloses no financial relationships relevant to the content of this presentation.

**References**