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The Role of Alternative Lengthening of Telomeres Mechanism in Cancer Cells and the Research Progress of Controllable Alternative Lengthening of Telomeres

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Abstract. The key to the unlimited proliferation of cancer cells lies in their ability to maintain telomere length, thereby evading the normal mechanisms of cellular senescence and death. Cancer cells mainly sustain or lengthen telomeres through telomerase activation and telomere elongation replacement mechanisms. The former is targeted at clinical trials of telomerase inhibitors (Imetelstat), which are currently ongoing. However, there are still many unsolved mysteries regarding the specific molecular mechanism of the Alternative Lengthening of Telomeres (ALT)pathway, which limits the development of anti-cancer drugs targeting this pathway. By reviewing relevant literature, this review will discuss the progress and new discoveries of this mechanism in recent years. The research conclusion indicates that although progress has been made in inhibiting key targets of the telomeric substitution elongation pathway (such as topoisomerase IIIα) and developing molecular tools, and a quality control mechanism has been discovered for ALT cancer cells, the specific delivery and toxicity management of targeted drugs remain challenges in clinical translation.

Keywords: ALT, Cancer cells, homologous recombination error correction mechanism, terminal protection, genetic engineering

1. Introduction

ALT stands for Alternative Lengthening of Telomeres Mechanism. It is a telomerase-independent mechanism that exists in approximately 10-15% of cancers (up to over 50% in osteosarcoma and up to 30% in glioblastoma) and also has a relatively high proportion in pancreatic neuroendocrine tumors) [1]. As one of the ways for cancer cells to acquire unlimited replication potential, ALT is more unstable compared to the traditional way in which cancer cells directly activate telomerase. However, it relies on the capacity of homologous recombination-mediated DNA repair, providing new insights into the regulation of cancer cell progression. In recent years, significant progress has been made in the research on the ALT mechanism. Although these studies have laid the foundation for clinical translation, the specific delivery and toxicity management of targeted drugs remain key issues to be addressed. This article will start with ALT recombination and, in combination with the error correction mechanism of homologous recombination, summarize and analyze the

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"controllable" difficulties of ALT [2]. By delving into the complexity of the ALT mechanism, this article aims to provide new strategies and ideas for cancer treatment.

2. The relationship between telomeres and cancer

According to the role of telomere length in cell carcinogenesis and tumor risk prediction, telomeres act as a natural protective barrier that prevents chromosome ends from being recognized as DNA damage signals, thereby avoiding the activation of the DNA repair system [3]. Understanding the existence of telomeres itself is actually a cancer suppressor mechanism. Therefore, throughout the entire life cycle of somatic cells, telomere length gradually shortens, which prevents unlimited cell replication and effectively restricts the proliferation of damaged cells. However, in most cancers, cancer cells will try every possible way to make telomeres regrow.

2.1. Telomere elongation substitution mechanism

As a specific telomere maintenance mechanism in certain cancers (such as osteosarcoma and neuroblastoma), the ALT pathway does not rely on telomerase but replicates telomere DNA through homologous recombination repair (HRR) [4]. Telomeres of AlT-positive cells typically exhibit extreme length heterogeneity (coexistence of very long and very short telomeres), frequent telomere sister chromatid exchanges, and abundant extrachromosomal telomeric DNA (such as circular telomere DNA, called t-circles). Extensive telomeric sister chromatid exchanges, along with abundant qualitative features, and large amounts of extracellular telomeric DNA (such as circular telomeric DNA, termed t-circles) (topoisomerase III DNA, such as circular telomeric DNA). They are called t-circles, so in most cases, these telomeres are in a low-damage state [5]. When a telomere becomes excessively short, it will use the DNA sequence of a nearby longer telomere as a template to replicate through the homologous recombination repair pathway, thereby using the DNA sequence of the telomere sequence on the template as a template to replicate through the homologous recombination repair pathway, and 'copy' the telomere sequence on the template to itself to achieve elongation.

2.2. Reasons that are difficult to control

STING pathway: It plays a significant role in inhibiting tumorigenesis. STING is an endoplasmic reticulum-related adapter that can transmit double-stranded DNA (dsDNA) signals sensed by cGAS, activate downstream kinase TBK1, and subsequently phosphorylate the transcription factor IRF3, driving the expression of type I interferons (IFNs) [6]. When telomere repeat DNA (ECTR) accumulates in the cytoplasm, it can be recognized by the cGAS-STING pathway and induce an immune response. However, Western blot analysis revealed that the STING protein was absent in the ALT cell line. Similarly, RT-PCR analysis revealed the absence of STING protein in ALT cell lines. Similarly, RT-PCR analysis showed a significant reduction in STING mRNA levels in ALT cells, which may be a necessary condition for the development of ALT cancer. Meanwhile, ALT cancer cells evade immune surveillance by inhibiting the STING protein, leading to abnormal telomere DNA exchange and chromosomal fusion. These fusion events trigger the amplification of oncogenes and genomic rearrangement, driving malignant transformation [7]. Meanwhile, Flynn's team's research indicates that ALT telomeres rely on the helicase SMARCAL1 to maintain telomere stability. When SMARCAL1 is absent, replication pressure causes double-strand breaks in DNA. The ends of the broken chromosomes are connected through non-homologous ends to form

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bicentromeric chromosomes, which are pulled by the spindle during mitosis to form "chromosome Bridges". Ultimately, random breaks lead to gene amplification or deletion, thereby triggering extensive genomic instability and further driving cancer progression. This is also the so-called "broken-fencing-bridge cycle" theory (first proposed by Barbara McClintock in corn research). Dilley et al. provided new insights into the initiation and maintenance mechanism of ALT in cancer cells through broken-induced telomere synthesis, especially through the BITS process. The following text will elaborate in detail.

2.3. Dysfunction of key regulatory proteins

The research by O'Sullivan's team has experimentally demonstrated for the first time that the deficiency of ASF1a/ASF1b proteins directly induces the ALT phenotype. As chaperone proteins for histones H3.1-H4, depletion of ASF1 leads to defects in telomeric nucleosome assembly, which in turn induces abnormal telomere DNA exchange between chromosomes (such as telomere sister chromatid exchange). Orphan nuclear receptors (COUP-TF1/2, TR2/4) are recruited to the telomere region through zinc finger protein 827 (ZNF827), inducing the formation of PML bodies and ALT activity (such as telomere DNA synthesis, and C-circle generation). These receptors synergize with the loss of function of the ATRX-DAXX complex (telomere inhibitory factor), significantly promoting the ALT phenotype. Studies have also confirmed that arsenic trioxide inhibits PML body formation by degrading PML proteins and effectively blocks tumor growth in ALT xenograft models (findings from the 2024 "Nucleic Acids Research" study). In ALT cancer cells, ECTR is supposed to activate the cGAS-STING pathway to induce interferon release, but in reality, the STING protein is inhibited, resulting in immune response failure. This immune escape protects cancer cells from growth inhibition and becomes a key mechanism in the development of ALT tumors [8].

3. Reorganization of the ALT pathway

The ALT pathway maintains telomere length through the homologous recombination (HR) mechanism. The recombination process involves abnormal exchange of telomere DNA, replication stress response, and the formation of specific nuclear structures. The essence of the ALT pathway is that cancer cells hijack the DNA repair mechanism to maintain telomeres. Its recombination process takes APB as the hub, synthesizes telomere DNA through BIR, and relies on helicases such as BLM to treat complex intermediates. Generally speaking, the recombination of ALT is partially associated with sister chromatid exchange (see Table 1 and Figure 1), and both rely on homologous recombination. Imagine if the error correction mechanism of normal somatic cells were applied to the recombination of ALT; would the regulation of the ALT pathway become traceable?

Table 1. The association of ALT recombination with normal somatic cells

Feature	Normal Homologous Recombination	ALT Telomere Recombination
Sequence Specificity	High: Requires strict sequence homology (homologous chromosome pairing)	Minimal: Telomeres are simple repeats (TTAGGG), prone to mispairing/ recombination with non-telomeric repeats (e.g., subtelomeric regions)
Error Risk	Low: Has error correction mechanisms (e.g., synaptonemal complex, Msh4/Msh5)	Very High: No spatial constraints, prone to illegitimate recombination (e.g., recombination between telomeres and internal genomic repeats)
Product Stability	Stable: Produces precise crossovers or gene conversions	Unstable: Leads to t-circle excision, telomere fusion, chromosome breakage

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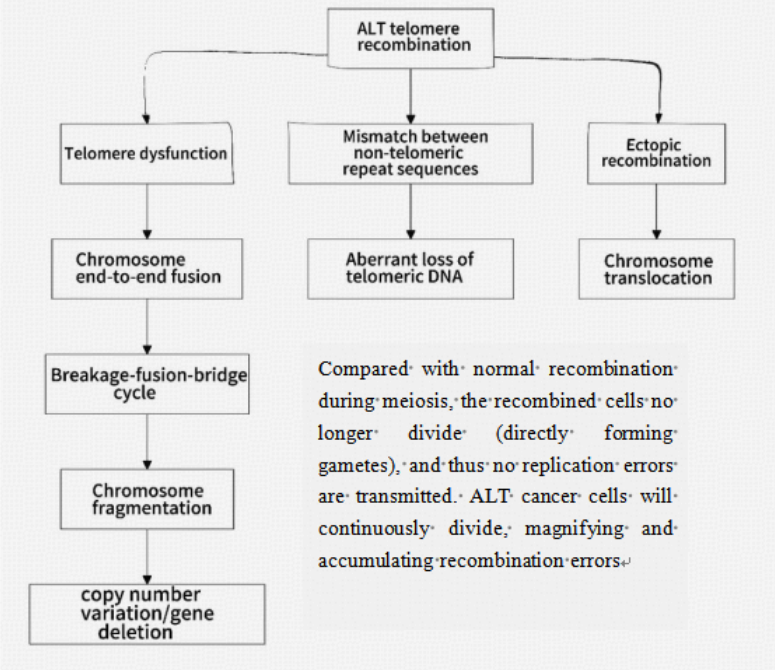

Figure 1. The causes of ALT causing cancer

4. Terminal protection of telomeres

In the 1990s, researchers such as Jack W. Szostak and Elizabeth H. Blackburn proposed the concept that telomeric sequences play a role in protecting chromosome ends. Telomere terminal protection refers to the mechanism by which telomeres (repetitive DNA sequences at the ends of chromosomes) prevent the ends of chromosomes from being recognized as DNA damaged, and avoid degradation and fusion by forming special structures (such as T-loops) and binding protective proteins (such as shelterin complexes). We know that the direct cause of the activation of the ALT mechanism is the absence of terminal protection, which activates the DNA repair system. That is to say, if this 'protective cap' can be restored, the ALT pathway will likely be inactivated. When recombination is needed, we can add this "cap" to this telomere section again. Suppose we temporarily open the "hat" and use some genetic engineering methods to make the cells meet the following three points: 1) Recombination occurs on sister chromosomes; 2) No nuclease cleavage; 3) No exposure of chromosome ends;Then it can meet the function of controllable and unlimited replication of cells.

5. The latest research progress of ALT

5.1. BITS process

Dilley et al. induced telomere double-strand breaks in ALT cells using the TRF1-Fok1 system. After induction, a tenfold increase in telomeric DNA synthesis was observed; this synthesis was unidirectional and continuous, capable of generating telomeric repeat sequences up to 20,000 base pairs in length. At the same time, after synthesis, it was observed that the characteristics of BITS were similar to those of breakage-induced replication in yeast, which serves as a telomere maintenance mechanism in yeast strains lacking telomerase.

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—Induction and maintenance of ALT: Further research is needed on how the ALT mechanism is induced and maintained, particularly in the 10–15% of cancer types that utilize the ALT pathway.

—Histone function: Disruption of histone function has been shown to induce ALT-like characteristics, suggesting a mechanistic link between telomere histones and the ALT mechanism.

—ATRX-DAXX complex: This complex mutation has a high recurrence rate in ALT tumors, but current studies have not explored how it leads to ALT, which will be an interesting research direction [9].

5.2. Research on BRD4

BRD4, as a telomere length regulator, was identified by shRNA screening. All four BRD4 inhibitors blocked telomere elongation in a dose-dependent manner. Some studies have shown [10]. BRD4 inhibitors have no direct effect on the enzymatic activity and processability of telomerase. Moreover, long-term treatment of mouse and human cells with BRD4 inhibitors resulted in significant telomere shortening, as well as increased numbers of chromosomal fusions and signal-free ends (SFEs). Clinical significance: BRD4 inhibitors are currently in clinical trials for various blood cancers. Telomere shortening may enhance their anti-cancer effects in clinical trials, but potential side effects on human patients with short telomere syndrome also need to be watched out for.

5.3. The role of TRIM24 in the ALT mechanism

TRIM24 identification: TRIM24(a chromatin acetylation reader protein containing bromine domains) was identified as a key regulator of replication stress response by BLOCK-ID technology. TRIM24 is recruited to telomeres through a p300/ Creb-binding protein (CBP)—dependent acetylated chromatin signaling cascade, organizing the assembly of AlT-associated PML bodies (APBs) and promoting the synthesis of new telomere DNA. Meanwhile, TRIM24 coordinates chromatin signaling in the ALT mechanism to maintain telomere stability.

—Clinical application prospects

Diagnostic value: TRIM24 may serve as a diagnostic marker for AlT-positive tumors.

Therapeutic target: Drugs targeting the TRIM24-p300/CBP signaling axis may represent a therapeutic strategy for ALT-positive cancers.

Technology promotion: The BLOCK-ID technology (in a human osteosarcoma U2OS cell line carrying 256 repeated LacO sequences, using the BirA-LacI fusion protein to achieve specific biotin labeling of proteins at blocked replication crosses) can be widely applied in replication stress-related research [11].

6. ALT and telomerase double-negative tumors

Double-negative prostate cancer (DNPC) is a specific subtype of prostate cancer characterized by the simultaneous absence of androgen receptor (AR) and neuroendocrine markers. This type of tumor often occurs after androgen deprivation therapy (ADT), shows significant resistance to traditional AR-targeted therapies (such as enzalutamide, abiraterone, etc.), and has a poor clinical prognosis. However, the cellular origin and molecular regulatory mechanisms of DNPCS have long been unclear.

The absence of a connection between KMT2C and the development of DNPC. The research team found through the analysis of clinical samples that. KMT2C gene mutations occur frequently in

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human DNPCS and are significantly associated with poor prognosis in patients. Tumors carrying KMT2C mutations or deletions are prone to transform into DNPC after ADT. Animal models have confirmed that Kmt2c gene knockout significantly accelerates tumor progression after ADT treatment and enhances resistance to AR-targeted drugs treatment strategy [12].

7. Conclusion

At present, research on the ALT pathway mainly focuses on the inhibition of key targets, the development of molecular tools, and clinical translation strategies. For example, targeting topoisomerase IIIα. Prashant Khandagale et al. found that knockdown of TOP3A can lead to inhibition of ALT cell growth, telomere abnormalities and formation disorders of APB (ALT-associated PML bodies). Targeting TOP3A to induce its cross-linking with DNA (TOP3A-DPC) can selectively kill AlT-positive cancer cells. Meanwhile, a recent study published in Nature revealed that ALT cancer cells actually possess a quality control mechanism that eliminates cells with excessive recombination errors. This indicates that even in such a chaotic system, life is still attempting to establish order. Although multiple studies have laid the foundation for clinical translation, breakthroughs are still needed in the specific delivery and toxicity management of targeted drugs. Rather than fantasizing that ALT can regulate recombination, it is better to develop drugs that simultaneously disrupt protective mechanisms and recombination accuracy (such as inducing overexpression of shelterin protein to "freeze" telomeres, or using CRISPR to insert specific marker sequences into telomere repeat sequences).

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