

A Comparative Study on TERT Overexpression and CRISPR Editing Cell immortalization Techniques

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Abstract

The boundary between cancerous and normal cell is thin. As cancerous cells are caused by mutations from the accumulation of DNA lesions, it is important to look into the process of cellular transformation to find ways to develop controlled cell immortalization. (Yoshioka, 2015) There are many methods of cell Immortalization. The methods focused in this paper are TERT(Telomerase reverse transcription) overexpression and CRISPR editing.

TERT overexpression is successful across different cell types, including specific blood cells, macrophages, and human kidney cells. (Huang et al., 2024) Each shows a high efficiency in transfection indicating a successful outcome of T-cell immortalization with minimal genomic alteration.

Emerging CRISPR editing approaches, including CRISPR-Cas9 genome editing and CRISPRa-mediated transcriptional activation, present a more controlled method of cell immortalization, avoiding insertional mutagenesis and providing controls and potential reversibility. (He et al., 2025; Zhao et al., 2020) Notably, it is the first time that a normal human T cell line is established. However, challenges remain, as the risk of off-target mutation and double strand break induced instabilities could occur. Both techniques demonstrate promising cell immortalization outcomes with TERT overexpression showing minimal genomic instability. CRISPR, as an editing tool, shows positive direction on the mobility of immortalization methods but its amount of negative effects on genomic stability remains unclear.

This review compares TERT overexpression and CRISPR-based immortalization with emphasis on genomic stability, an aspect typically discussed separately in existing studies. By synthesizing findings, this paper evaluates which approach currently offers the most balance between immortalization and genomic stability.

Introduction

In 1961, cells were discovered to have a limit in division. (Hayflick and Moorhead, 1961) Later understood to be closely associated with progressive telomere shortening, studies regarding telomere extension as a method for immortalization emerged.

CRISPR-Cas9, discovered in 2012 by Jennifer Doudna and Emmanuelle Charpentier, provided a brand new route into genetic editing. However, its ability to target specific genes is not limited to gene editing but also cell immortalization.

Comparing the two immortalization strategies. The primary result is the successful extension of cellular lifespan beyond unmodified primary cells, while maintaining non-tumorigenic properties. However, the degree of chromosomal stability, telomerase activation, and early-passage variability differ among the techniques.

Methods

The studies selected for this review were based on three criteria: the studies are published in peer-reviewed journals, relevance to human primary cell immortalization, and experimental evaluation of immortalization techniques with genomic stability assessments.

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With exceptions of earlier reviews from before the 2000s as backgrounds on telomerase overexpression, Crispr technologies, and genomic instability during immortalization.

Results

TERT-immortalized cells demonstrate great chromosomal stability and extend proliferation without malignant behavior. In contrast, as the first method to establish normal human T cell lines, CRISPR-based offers a controlled strategy that avoids random viral insertion events. (He et al., 2025; Zhao et al., 2020)

Currently, TERT overexpression appears to provide a more stable strategy than presently available CRISPR-based immortalization studies. However, CRISPR-based immortalization allows unprecedented precision and the possibility of immortalizing clinically important primary cells such as T cells. With continued refinement of CRISPR technologies, particularly the reduction of CRISPR-induced DNA breaks, it may eventually surpass existing methods.

Discussion

The findings agree with earlier research showing that hTERT can extend cellular lifespan while maintaining normal chromosome numbers (Bodnar et al., 1998).

In contrast, CRISPR provides greater control over specific genes and can be adapted to difficult-to-immortalize cells such as primary T cells (Zhao et al., 2020; He et al., 2025).

Overall, the comparison suggests a trade-off between stability and flexibility. With TERT primarily preserving telomeres, CRISPR provides greater control over the mechanisms of immortalization.

Conclusion

TERT overexpression is associated with reduced genomic instability by maintaining telomere length. CRISPR, on the other hand, is a useful tool for controlled immortalization.

Both of them are immortalization techniques but based on different concepts: hTERT overexpression is telomerase-based and CRISPR includes both genome editing and transcriptional activation based approaches.

By understanding all of these immortalization methods, it will be possible to merge and develop even newer techniques which could provide further help in advancements in the medical fields such as regenerative medicines.

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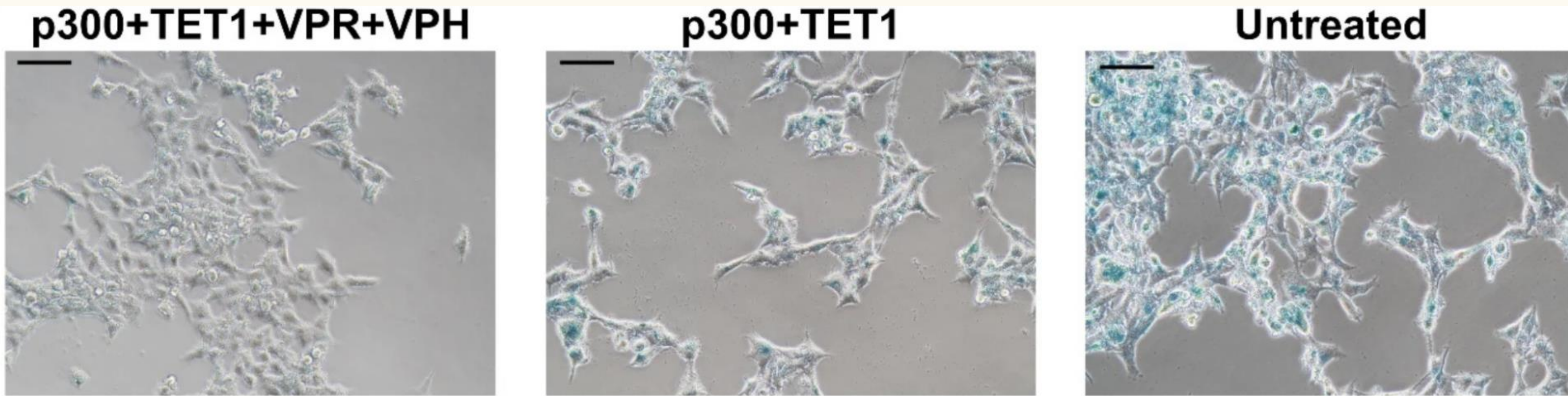
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senescence-associated β-galactosidase staining of HEK293FT cells.
- Senescent cells are expressed by the blue dye staining