論文の内容の要旨

Evaluation of Telomerase Reverse Transcriptase Localization during Cell Death Process by Live-Cell Imaging

(生細胞イメージングを用いた細胞死過程における テロメラーゼ逆転写酵素 TERT の局在評価)

江端拓志

Telomerase reverse transcriptase (TERT) is a protein subunit of the telomerase complex, which elongates the telomeric repeat sequences at chromosomal ends and prevents telomere loss. Several studies have shown that TERT is localized not only in the nucleus but also in mitochondria, which lack telomeric regions. Oxidative stress, which eventually leads to cell apoptosis, has been reported to increase TERT localization in mitochondria, raising the possibility of a noncanonical role of mitochondrial TERT in apoptosis beyond its telomere elongation function. Mutagenesis studies have proposed that mitochondrial TERT induces apoptosis. However, TERT overexpression has been reported to increase TERT in mitochondria and cell survival after oxidative stress, suggesting that mitochondrial TERT suppresses apoptosis. These reports, while conflicting, have shed light on the possibility that mitochondrial TERT regulates apoptosis.

Previous immunofluorescence- and flow cytometry-based measurements only provide information on cell death and TERT localization at specific times. However, oxidative stress induces apoptosis through several different pathways. Even within the same pathway, the response time to apoptosis-inducing stimuli varies among cells. Thus, directly testing the relationship of mitochondrial TERT and apoptosis requires tracking the two factors over time. Here, I combined imaging-based dead-cell detection methods with live-cell fluorescence imaging of TERT to directly assess the relationship between the mitochondrial localization of TERT and apoptosis of individual cells.

To visualize TERT distribution in individual cells, I inserted mVenus into TERT,

and in preparation for the visualization of TERT localization, I assessed the effect of the insertion on the telomerase activity and localization pattern of TERT. Conventionally, epitope tags or fluorescent proteins are conjugated to TERT at the N- or C-terminus. However, TERT has a mitochondrial targeting signal at the N-terminus and an essential domain for telomerase enzymatic activity at the C-terminus, suggesting conjugation at either location could affect TERT function. Instead, I inserted mVenus between A67 and A68 (hereafter mVenus-TERT), which I considered an ideal location because it is far from the reaction site of the telomerase complex and telomerase co-factors. I evaluated the telomerase activity and localization of mVenus-TERT. As expected, mVenus insertion did not interfere with the telomerase activity and mitochondrial localization of TERT. Accordingly, I used mVenus-TERT to visualize TERT during apoptosis by live-cell imaging.

Using mVenus-TERT, I performed the following three simultaneous fluorescence measurements: dead cells visualized by SYTOX Blue staining, mitochondria visualized by MitoTracker Deep Red FM, and TERT visualized by mVenus. The time until apoptosis was calculated as the time when the fluorescence intensity of SYTOX Blue reached a specified threshold. Also, I calculated colocalization metric, MCC of TERT with mitochondria, from the fluorescence intensity at each time point (Fig. 1).

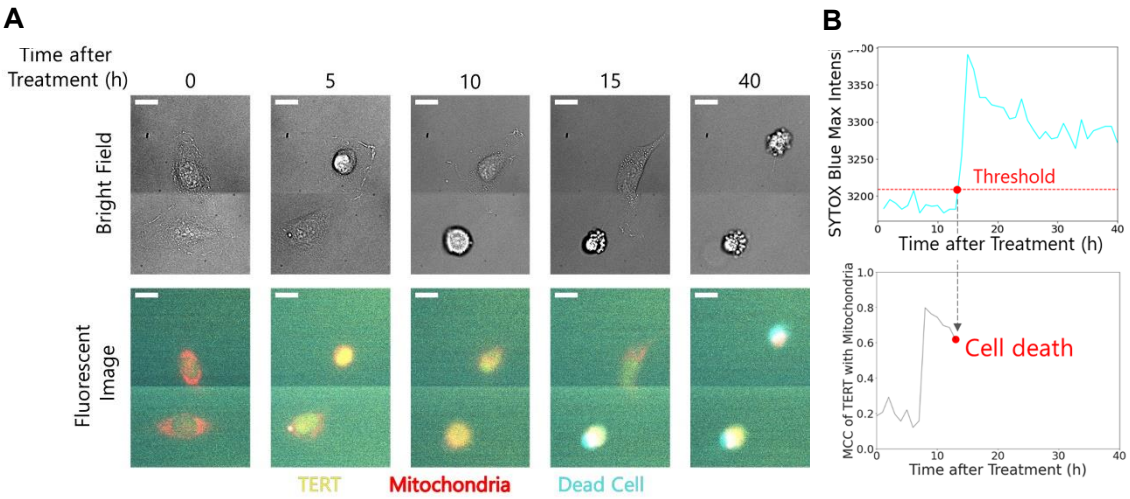

Fig. 1 Simultaneous live-cell tracking of cell death and TERT localization.
A. Representative live-cell images of HeLa cells expressing mVenus-TERT constructs. Yellow, mVenus fluorescence; red, MitoTracker Deep Red FM fluorescence; cyan, SYTOX Blue fluorescence. Cells were treated with 267 μM calcium percarbonate for 3 h before the imaging. Scale bars, 20 μm. **B.** Representative time-course traces of SYTOX Blue fluorescence intensity and MCC of TERT with mitochondria of the cell in the bottom panel in A. Cells were determined as positive after SYTOX Blue fluorescence intensity exceeded the threshold.

From the live-cell tracking, I obtained the time-course plot of the MCC of TERT with mitochondria after oxidative stress in cells expressing mVenus-TERT. This plot demonstrated that cells with high MCC did not survive and all the surviving cells showed low MCC (Fig. 2A). This observation of the mitochondrial localization of TERT after oxidative stress is consistent with previous reports. In the present study, the cells experienced 3 hours of oxidative stress before tracking, and cells with high MCC appeared in the first frame of the tracking. Therefore, cells that showed an accumulation of mitochondrial TERT had their fate determined within 3 hours of the oxidative stress treatment. Additionally, I found a positive correlation between MCC at the start of the imaging and time until apoptosis in cells expressing mVenus-TERT (Fig. 2B). This observation indicates that cells expressing mVenus-TERT with high MCC tend to take longer to undergo apoptosis. From these results, I established a model for the role of mitochondrial TERT in apoptosis (Fig. 3).

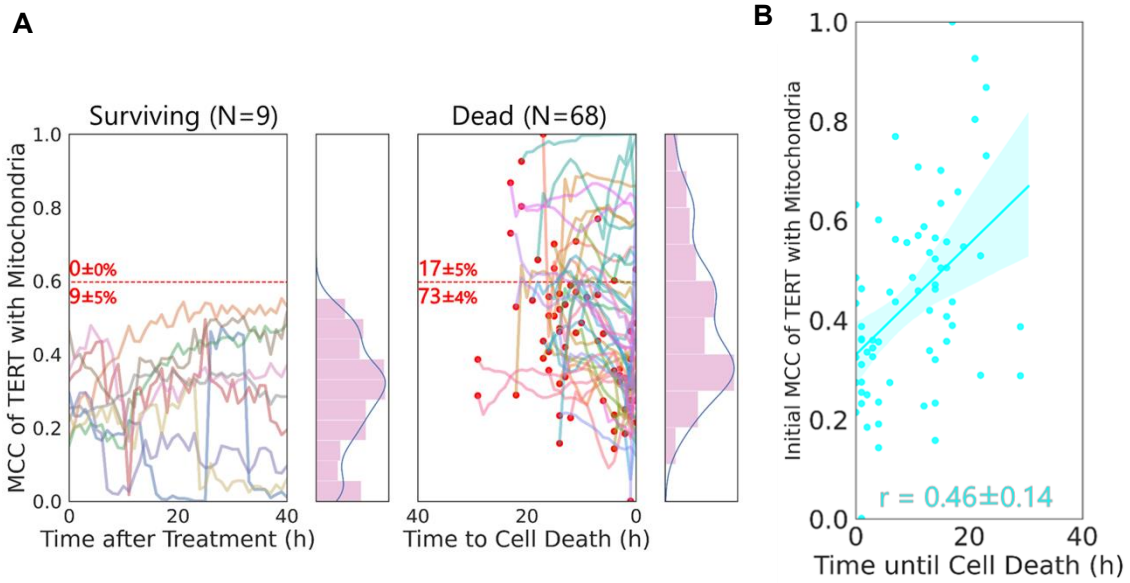

Fig. 2 Live-cell tracking revealed the effects of mitochondrial TERT in apoptosis.
A. Time-course plot of MCC of TERT with mitochondria in each cell. A histogram and KDE plot of all MCC are shown. Plotted values are the mean values per 5 frames. For dead cells, 0 in the x-axis represents the moment the cells died. Red dots show MCC at the beginning of the observation. Red numbers show the percentage (mean ± SEM from 3 independent experiments) of cells whose initial MCC was above or below the threshold (top 2.5% of surviving cells) represented by the red dashed line. **B.** A scatter plot and regression line between the initial MCC of each dead cell and time until the cell death. Translucent bands around the regression line represent 95% C.I. r, Pearson's correlation coefficient (PCC), shows the mean ± SEM from 3 independent experiments. N=68 cells.

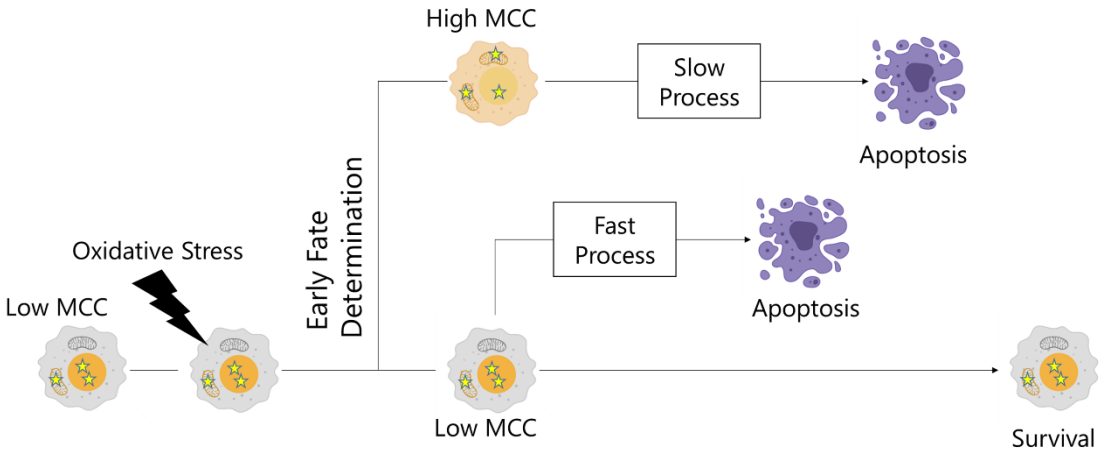

Fig. 3 A new model for the roles of mitochondrial TERT in apoptosis.
This figure was created with BioRender.com.

Based on these results, I propose a new model that integrates the seemingly contradictory data from previous studies for the role of TERT in apoptosis (Table 1). I speculate that there are two stages after oxidative stress. In the first stage, which is immediately after oxidative stress, mitochondrial TERT promotes apoptosis. Because mitochondrial TERT is one of the earliest markers known to date for cells undergoing apoptosis and other reports found mitochondrial TERT induces apoptosis, I propose a model in which this accumulation predetermines apoptosis. However, in the second stage, mitochondrial TERT delays the apoptotic process. This delay can explain why previous studies found that mitochondrial TERT suppresses apoptosis. Thus, our model includes the opposing hypotheses about the involvement of mitochondrial TERT in apoptosis.

Table. 1 A model with two stages integrates the controversy on the roles of mitochondrial TERT in apoptosis.

Stage	Mitochondrial TERT Role	Previous Studies
1. Fate Determination	Predetermines apoptosis	Induces apoptosis
2. Apoptotic Process	Delays apoptotic process	Suppresses apoptosis