



Department of Biotechnology  
Ministry of Science and Technology  
Government of India

**DBT**



National Institute of  
Advanced Industrial Science  
and Technology

**AIST**

**DBT - AIST International Laboratory**  
**for Advanced Biomedicine**

**DAIILAB**

**Classroom for Advanced & Frontier Education**

**CAFE**

# DAI LAB - CAFE

## Series - 14

Date and Time - May 19, 2016 (12:30~13:30)

Venue - Central 6-11 (2F) Meeting Room # 216

Speaker – **Zeenia KAUL**

Affiliation – Dept. of Mol Virology, Immunol & Med Genetics, The Ohio State University, Ohio, USA

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## Five dysfunctional telomeres mark cellular senescence in normal human diploid fibroblasts

Replicative senescence is associated with progressive telomere shortening in primary fibroblasts, and its onset coincides with a telomere-specific DNA damage response (DDR). Cellular immortalization requires activation of a telomere length maintenance mechanism (TMM) - telomerase or Alternative Lengthening of Telomeres (ALT). Telomere dysfunction initiates a DDR at chromosome termini that can be visualized by the co-localization of DDR proteins and telomeres in telomere-dysfunction induced foci (TIFs).

To study spontaneous telomere dysfunction, we developed a sensitive technique to detect metaphase TIFs (meta-TIFs) by analyzing cyto-centrifuged metaphase spreads by a combination of immunostaining of  $\gamma$ -H2AX (a robust DDR marker) and telomere Fluorescent *In Situ* Hybridization (FISH). We cultured normal diploid human breast fibroblasts obtained from five healthy donors. The cells were passaged from early population doublings (PDs) until replicative senescence, and the meta-TIF assay was performed at four points on the growth curve. We found that a small number of meta-TIFs are present in young dividing cells and the number of meta-TIFs increases with increasing PDs. Of note, the presence of ~5 dysfunctional telomeres was associated with the onset of replicative senescence in normal primary breast fibroblast cells. Analysis of meta-TIFs revealed two distinct forms of TIFs: (i) chromosome type dysfunction (involving both chromatids of a metaphase chromosome) and (ii) chromatid type dysfunction (single chromatid). Single-chromatid type TIFs accounted for the majority of the dysfunctional telomeres in primary breast fibroblasts. Combining chromosome-orientation FISH with the meta-TIF assay showed that the majority of chromatid-type TIFs originated from both the leading and lagging strand replicated telomere. We also used the meta-TIF assay to analyze a large panel of immortalized and cancer derived cell lines for spontaneous telomere dysfunction and found a large number of spontaneously dysfunctional telomeres (i.e. meta-TIFs) in cells lacking wild-type p53 activity, where telomeres are maintained either by a very low level of telomerase activity or by ALT. In immortalized cells with functional p53, the number of meta-TIFs was found to be below the threshold associated with onset of senescence in normal cells.


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**SERIES - 14**

**Thanks for participation!**

**Speaker: Dr. Zeenia KAUL**

**Topic: Spontaneous telomere dysfunction in immortalized and normal human cells**

**Date: 19<sup>th</sup> May 2016 (1230-1330 hours JST)**

**Host: AIST, Japan**

**National Institute of Advanced Industrial Science & Technology, Japan**



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