Telomere Length Measurements of Human and Mouse Cells by Fluorescence in situ Hybridization

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Telomeres are located at the ends of chromosomes and consist of tandemly repeated DNA sequence (5'-TTAGGG-3') associated with proteins. Telomeres confer stability to the chromosome termini, i.e. protect the chromosome ends from being recognized and processed as double strand breaks. Telomere dysfunction results in genetic instability and is implicated in aging and cancer. A sensitive method to determine the telomere length in single metaphase cells is the so-called Quantitative Fluorescence in situ Hybridization technique (Q-FISH). Telomeres are visualized by means of a fluorescent labeled PNA probe as shown in Fig. 1. The signal intensity is directly proportional to the telomere length [1] and is quantified from digital microscopy images.

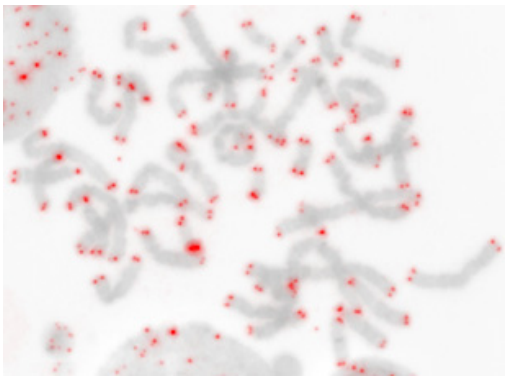

Fig 1: Typical Q-FISH image: Telomere sequences of a human metaphase spread stained with a Cy3-conjugated PNA probe (red spots, focus stack image). Chromosomes are counterstained with DAPI (gray).

Recently we set up the Q-FISH method and characterized human and mouse primary cells and established cell lines used in current radiobiological studies: human cells include primary lymphocytes, glioblastoma cells (line LAN1-WT) and embryonic stem cells (line H9); mouse cells include primary bone marrow cells, embryonic fibroblasts and embryonic stem cells (line D3). In each case >40 metaphases were analyzed.

Examples of chromosomes with stained telomeres are shown in Fig. 2. Analysis of the data clearly demonstrated that the telomeres of human cells are much shorter in comparison with mouse cells (Fig. 3). Moreover, in both species embryonic stem cells had longer telomeres than somatic cells. These observations are consistent with published data [2-4]. In subsequent studies we will use this method to uncover the relationship between telomere length and radiation induced chromosomal instability.

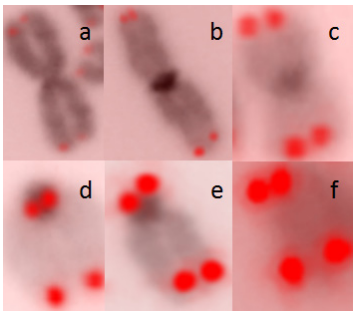

Fig 2: Increase in telomeric Cy3-signal intensity reflecting telomere length: (a) human glioblastoma cells (hGlio), (b) human lymphocytes (hLymph), (c) human embryonic stem cells (hESC), (d) mouse bone marrow cells (mBM), (e) mouse embryonic fibroblasts (mEF), (f) mouse embryonic stem cells (mESC); (b,d) are primary cells, while the others are established cell lines.

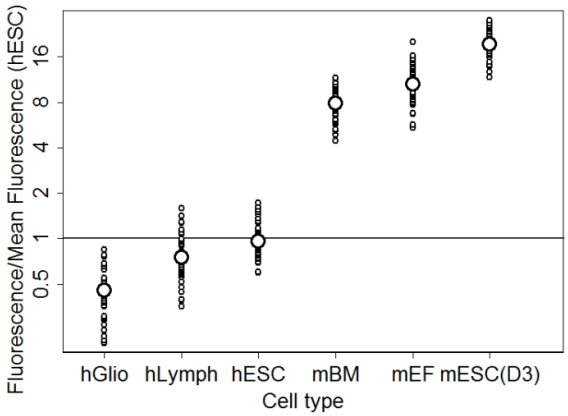

Fig. 3: Fluorescence measures of Cy3 telomere-stained metaphases. Data are normalized on the mean fluorescence of hESC. Abbreviations as in Fig. 2.

References

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