CHROMOSOMAL ANALYSIS OF INDUCED PLURIPOTENT STEM CELLS DERIVED FROM SENESCENT CELLS OF ELDERLY PERSONS

Context: Reprogramming of adult somatic cells into induced pluripotent stem cells (iPS) provide a unique opportunity to generate patient-specific stem cells with potential application in regenerative therapies and without the ethical concerns of human embryonic stem cells (hES).

Objective: important question persist about the safety of iPS, their chromosomal stability and their propensity to form tumor, in particular for iPS derived from senescent cells.

Patients and Methods: In order to assess the chromosomal consequences of reprogramming process, we performed classical and molecular cytogenetic analysis on 4 iPS lines generated from 74- and 96-years-old men’s fibroblasts. Cytogenetic analysis was carried out using standard methods and R-banding. FISH assays with both centromeric and painting probes were used for chromosomal detection on interphase nuclei. In three iPS lines, these investigations were completed by an analysis of the telomere sizes since senescence is characterized by telomere shortening. We measured in situ the length of telomere repeat domains using PNA pantelomeric probes and Metasystems image analyzer software Isis.

Results: A minimum of 20 metaphase spreads and 100 interphase nuclei per cell line were analyzed. The 4 cell lines exhibited normal karyotypes. The Isis software was used to quantify the fluorescence intensity of telomeres from at least 20 metaphases and nuclei from each cell line. In the 3 iPS lines, we observed a significant increase in telomere length, when compared with their parental fibroblasts and control hES. These data were confirmed by telomere restriction fragments analysis.

Conclusion: The use of iPS in regenerative medicine is of particular interest in the context of age-associated disorders, but cytogenetic and genetic screening of iPS need to become standard practice before these cells might be used clinically.