TELOMERE AND RADIOSENSITIVITY OF INDIVIDUALS

TELOSENS

PROJECT SUMMARY

Contract: FIGH-CT-2002-00217

1st January 2003 to 31st December 2005

I - INTRODUCTION

Risk estimate of radiation-induced cancer in human population is based on epidemiological data. Most extrapolations are limited by the lack of understanding of the mechanisms responsible for radiation carcinogenesis. The acquisition of knowledge on common genetic factors that might determine inter-individual differences in low dose cancer risk encounters similar limitations. During TELORAD Project (Feb 2000 - Jan 2003), evidence was accumulating that telomeres may be involved in cellular and organismal responses to IR. Mice lacking functional telomerase are radiosensitive. Ataxia telangiectasia, Fanconia patients are radiosensitive and show altered telomere maintenance. The major aim of TELOSENS consortium was to work on a goal oriented project to be able to give a precise answer to the following question:

Is telomere heterogeneity a parameter of individual radiosensitivity for short term effect?

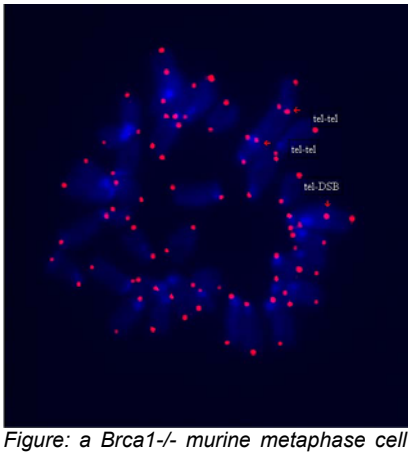


Figure: a Brca1-/- murine metaphase cell after the CO-FISH procedure. The presence of telomeric fusions (Tel-tel) and interaction between telomeres and broken chromosomes (tel-DSB) represent signs of telomere dysfunction.

II - OBJECTIVES

Telomeres are natural chromosome ends. The proteins involved in telomere maintenance play a role in the distinction between radiation-induced DNA breaks which are subject to repair and telomeres.

The aims of TELOSENS are :

1) To focus on the role of telomere heterogeneity (length, proteins, repair…) in radiosensitivity of individuals :

- To determine if yes or no telomere heterogeneity is a parameter that could modify risk quantification for short-term effect after irradiation.
- To test if telomere heterogeneity could play a role during radiation-induced cellular transformation.

2) To know if we need further to address the question of the importance of telomere heterogeneity in the individual susceptibility to develop a cancer as long term effect of radiation exposure.

III - MAIN ACHIEVEMENTS

1. Ligase III : a role in a new NHEJ pathway to repair DNA double strand breaks ?

Chromosome fusions are observed with high frequency in cells with defects in components of the standard NHEJ pathway of DNA double strand break repair which suggests the operation of an alternative repair pathway that is capable of joining together eroded telomeric sequences when the canonical pathway is inactivated. The results presented suggest that **DNA ligase III** may be a bona fide component of DNA DSB repair by NHEJ. It was investigated in detail the possibility that **backup pathways of NHEJ** exist that may gain prominence in NHEJ mutants and may cause the genomic and telomeric instability observed. These pathways of end joining could contribute significantly to the ability of cells of cope with radiation damage and therefore to their radioresistance to killing. However, since they are error prone, they contribute to the development of cancer as well as to the fusion of chromosomes, particularly when telomeres erode.

2. Link between telomere maintenance & DNA damage response mechanisms, and their role of individual radiosensitivity. Insights on action of BRCA1 gene.

Results within TELOSENS provide additional support for the link between telomere maintenance and DNA damage response mechanisms. One most significant finding is the potentially direct involvement of BRCA1 in telomere capping function. BRCA1 is a known tumor suppressor gene. Telomere maintenance also constitutes a powerful tumor suppressor mechanism. BRCA1 defective cells may, therefore, have failures in two tumor suppressor mechanisms. The exact mechanisms through which BRCA1 exerts its tumor suppressor properties are not fully understood. It is now of interest to investigate the possibility that BRCA1 may exert, at least in part, its tumor suppression through effects on telomere maintenance.

3. Double strand break repair functions and gene amplification

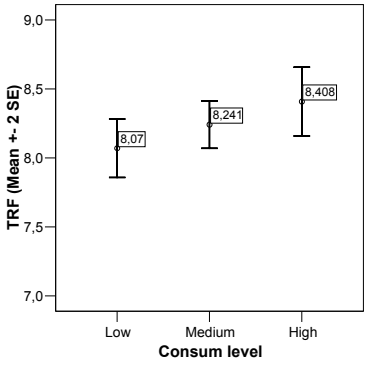
It is known that DNA-PKcs is a key protein for the repair of DSBs through the NHEJ pathway and that DSBs can initiate gene amplification. The results obtained by two TELOSENS partners indicate that in human cells, an **impairment in DNA-PKcs function** increases the proneness to DNA amplificaton. Promiscuous recombination events between broken ends which are not immediately and correctly rejoined could **trigger the amplification process**. Alternatively, a defect in DNA-PKcs could cause an increase in gene amplification by altering the equilibrium between NHEJ and HR.

4. Genomic instability and telomere length : radiation sensitivity of human mammary epithelial cells in culture

Human mammary epithelial cells (HMECs) derived from normal breast tissue from three women subjected to spontaneous reduction was analysed. The karyotype evolution of HMECs was followed by means of an exhaustive cytogenetic analysis. Most importantly, **sister chromatid fusion** was identified as the first event in generating genomic instability in HMECs. During HMEC growth, double strand breaks are generated by both fused chromosomes as well as individual chromosomes with fused chromatids entering **Breakage-Fusion-Breakage cycles**. These broken extremities are able to join other broken ends or eroded telomeres, producing massive instability at the latter passages of the cell culture. Evidence is accumulating that telomeres may be involved in cellular as well as organismal responses to ionizing radiation. **Dysfunctional telomeres** joined to radiation-induced DSBs in HMECs were identified, providing the first conclusive evidence of this type of aberration in primary human cells.

5. Telomeres modulate radiation response in vitro and in vivo - Vegetable intake prevents excessive telomere shortening.

One TELOSENS partner studied whether cells from individuals with long telomeres are protected against radiation-induced chromosome damage in a study involving 200 individuals of the same age. This partner found less radiation-induced chromosome damage in the subgroup of individuals with long telomeres. To their knowledge, this is the first study proving a **relationship between human telomere length and radiation-induced chromosome fragility** indicating that long telomeres protect from radiation induced DNA damage even. This effect was only observed when cells are irradiated at G1 phase of the cell cycle but not in G2 phase suggesting that the modulating effect of telomere length on radiosensitivity is cell cycle dependent. This study also provides **a molecular link between diet and aging** suggesting that high vegetable intake protects telomeres from an excessive shortening.



relation between between telomere length (TRF terminal restriction fragment) and level of fresh vegetable intake.

6. Effects of telomerase and telomere length on epidermal stem cell behaviour

In the past, it has been shown by one team involved in TELOSENS that mice with critically short telomeres are highly sensitive to ionizing radiation, i.e. show increased chromosomal instability upon gamma irradiation. More recent results shed light on the mechanism underlying telomere-driven radiosensitivity. The same team has determined the role of both telomerase and telomere length on epidermal stem cell behavior. In particular, mice with critically short and **dysfunctional telomeres** showed **a defective mobilization of epidermal stem cells**, which anticipated the fact that these mice

are resistant to skin tumorigenesis protocols and show a premature aging of the skin. In turn, mice that **over-expressed telomerase** in the skin showed **an augmented stem cell mobilization**, also anticipating the fact that these mice are more prone to tumorigenesis.

7. Roles of telomeric chromatin in malignant transformation.

Recent evidence has associated telomere instability to oncogenesis but the mechanisms and the significance for human cancers remains elusive. To explore the ability of **telomere dysfunction** to promote neoplastic transformation in defined human cellular systems, one TELOSENS team has developed human cellular systems to study the role of TRF2 in tumorigenesis and have shown that TRF2 dysfunction can trigger some of the steps involved in tumorigenesis. **For the first time has been established a causal relationship between telomere instability and transformation of human cells**. Relevant to radioprotection is the observation that, for a given cellular system i.e. immortalized fibroblasts, irradiation does not appear to contribute to transformation while telomere dysfunction does. This comparison is valid since approximately the same number of chromosome breaks and rearrangements were generated in either procedure. Therefore, telomere dysfunction appears to be more prone to trigger oncogenic events than gamma-irradiation, suggesting the existence telomere-specific events leading to transformation. A hypothesis is that telomere dysfunction **triggers an epigenetic reprogramming of the cell**, which renders it more prone to acquire oncogenic properties.

8. The adult mesenchymal stem cell : a target for neoplastic transformation.

One TELOSENS team has shown for the first time that the **adult human mesenchymal stem cell** is a target for transformation. They have identified by a phenotypic screen the key steps in adult stem cell transformation, including telomerase expression, cell cycle deregulation and differentiation defects. This study highlights the importance of well define reagents for biotechnology applications such as tissue engineering as well as defining basic concepts in cancer stem cell biology

IV - DISCUSSION OF RESULTS

Collectively, TELOSENS results provide support for the hypothesis that that telomere maintenance mechanisms and mechanisms of DNA damage response are tightly linked. A direct prediction from this hypothesis is that telomere maintenance may serve as a useful indicator of individual radiosensitivity. Further research is required to test this link more stringently.

The relationship between human telomere length and radiation-induced chromosome fragility shows that long telomeres protect from radiation induced DNA damage indicating that "yes" telomere heterogeneity is a parameter that could modify risk quantification for short-term effect after irradiation.

TELOSENS results show that telomere dysfunction plays a role during cellular transformation and could be a major player in radiation-induced cell transformation.

Up to date, most of the experimental studies evaluating the risks facing people exposed to ionizing radiation have been carried out in human lymphocytes. These cells derive from cell precursors that have telomerase activity. During TELOSENS project evidence accumulated that telomeres may be involved in human cellular (lymphocytes, fibroblasts, mesenchymal cells, mammary epithelial cells) as well as organismal responses to ionizing radiation. *The major mechanism underlying this increased chromosomal sensitivity is the presence of chromosomes with shortened telomeres that, by being used as substrates for illegitimate repair, join onto radiation-induced DNA double strand breaks thus interfering with the correct rejoining of the broken ends and challenging the dose effect response. All these results reinforce the potential key role of telomere maintenance within each level of organization (length, capping, chromatin structure) in the individual susceptibility to develop a cancer as long term effect of radiation exposure.*

V - BOXES

Box 1 : Examples of technical breakthroughs

1. **Halosperm**, a simple procedure to determine sperm DNA fragmentation, has been established. The integrity of sperm DNA is indeed being recognized as a new parameter of semen quality and a potential fertility predictor. However, it was not so far assessed as a routine part of semen analysis. A new improved technique to determine sperm DNA fragmentation has been established by a TELOSENS partner for human spermatozoa, being called the **Sperm Chromatin Dispersion (SCD) test**. This is a simple, fast, accurate and highly reproducible method for the analysis of sperm DNA fragmentation. It is commercialised as a kit under the name Halosperm.

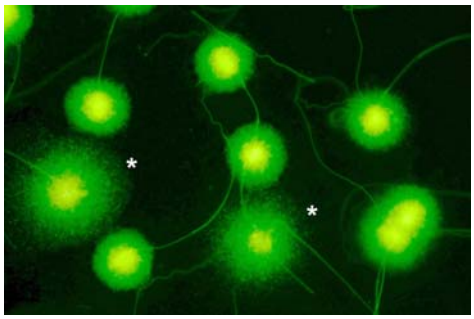
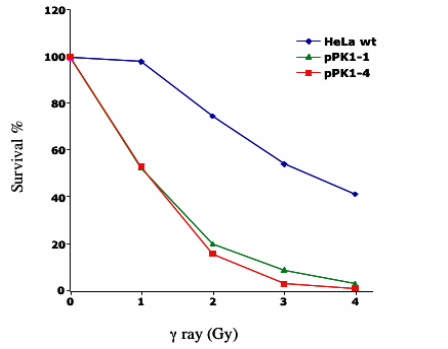


Figure: Mouse sperm cells processed by the specific variant of the SCD test, observed under fluorescence microscopy after SYBR Gold staining. Those sperm nucleoids containing fragmented DNA are indicated by asterisks

2. Human cell lines in which DNA-PKcs expression is constitutively inhibited by **RNA interference** have been produced. In two clones obtained from HeLa cell lines transfected with the same inhibiting oligonucleotide homologous to the DNA-PKcs mRNA, DNA-PKcs protein was efficiently inhibited. The cell lines obtained from the two clones are hypersensitive to γ-irradiation (*Figure right*) indicating that stable cell lines with a DNA-PKcs deficient phenotype by RNA interference were obtained.



3. A TELOSENS team has set up the technology to study the tridimensional architecture of the human interphase nuclei from the telomere perspective by **3D-FISH and confocal microscopy**.

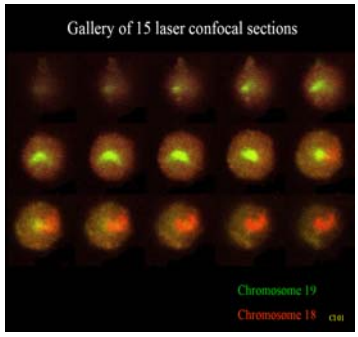


Figure: as internal control the architecture of chromosome 19 and 18 was studied by 3D-FISH as it is known that chromosome 19 has an internal position whereas chromosome 18 is peripheral in the nucleus

Box 2 : Major scientific breakthroughs during TELOSENS

- **DNA ligase III** may be a bona fide component of DNA DSB repair by alternative NHEJ pathway that is capable of joining together eroded telomeric sequences that may cause genomic and telomeric instability.
- Accelerated telomere shortening in **FA (Fanconi Anemia)** patients is not due to a role of FANCG at telomeres but **rather a consequence of the disease,** suggesting that telomerase-based therapies could be useful prophylactic agents in FA aplastic anemia, by preserving their telomere reserve in the context of the disease.
- A study on cells from 200 individuals of the same age proved for the first time a relationship between **human telomere length and radiation-induced chromosome fragility** indicating that long telomeres protect from radiation induced DNA damage events.

- **BRCA1**, a known tumor suppressor gene, is potentially directly involved in telomere capping function.
- Dysfunctional telomeres joined to radiation-induced DSBs were identified in **Human Mammary Epithelial Cells**, providing the first conclusive evidence of this type of aberration in primary human cells
- Dysfunction of **TRF2**, a protein involved in the telomere binding complex, can trigger some of the steps involved in tumorigenesis. Thus for the first time has been established **a causal relationship between telomere instability and transformation of human cells**.
- Novel mechanisms of telomere maintenance were identified. Evidence for the implication of **epigenetic mechanisms** in the regulation of mammalian telomere length and function was provided, showing the interplay between heterochromatic features and telomere length regulation.

Box 3 : Dissemination of results

TELOSENS has produced **83 publications**, which contribute to the 16 scientific deliverables. Among these publications, 78 (72 have already been published) are in peer-reviewed papers, with a mean **impact factor of 7,31**. Other publications are 2 articles in proceedings of conferences and 3 book chapters. 1 patent, along with 6 international extensions, concerning a novel procedure to determine sperm DNA fragmentation has been granted.

VI - TELOSENS CONSORTIUM

TELOSENS has brought together **12 European laboratories** from six countries (France, Italy, Spain, Germany, UK, Denmark) thus creating a critical mass of scientists capable of answering some outstanding questions in the field of telomere biology. This emerging field of research aims at understanding the role of telomeres, specialised structures at chromosome end, in aging and tumorigenesis. TELOSENS was coordinated by **Dr Laure Sabatier** from CEA (French Atomic energy Commission) Research Center in Fontenay-aux-Roses, France.

The overall question addressed by TELOSENS was divided into three work packages focused on specific aspects of telomere biology.

WP1-**Telomere maintenance, radio-sensitivity and DNA double-strand break repair in mammalian systems**:	The aim of this WP was to characterize the mechanisms underlying telomere-driven radiosensitivity, focussing on the interplay between telomere maintenance and DNA repair.
WP2-**Telomeres and radiosensitivity of individuals**.	The general purpose of this WP is to identify if and how telomere maintenance (length, heterogeneity, proteins...) modulates the radiosensitivity among individuals.
WP3-**Telomere, telomerase and Chromosomal Instability during radiation-induced cellular immortalization.**	This WP explores the ability of telomere dysfunction to promote neoplastic transformation in defined human cellular systems.

Partners :

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