

Extra Views

The Frequency of Homologous Recombination in Human ALT Cells

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ALT, homologous recombination, double strand break, telomerase, telomere length, immortalization, cancer

ABBREVIATIONS

ALT	alternative lengthening of telomeres
GFP	green fluorescence protein
DSB	double strand break
APB	ALT associated PML body
PML	promyelocytic leukemia
NHEJ	non-homologous end joining
BIR	break induced replication
MMR	mismatch repair

ABSTRACT

Instead of telomerase, some immortal cells use the alternative lengthening of telomeres pathway (ALT) to maintain their telomeres. There is good evidence that homologous recombination contributes to the ALT mechanism. Using an inducible GFP reporter system to measure the frequency of homologous recombination, we asked whether or not ALT cells exhibited a general change of the recombination machinery. Our results show that the frequency of homologous recombination for non-telomeric sequences in ALT cells is identical to that in telomerase positive cells, irrespective of whether the reporter was present at an intra-chromosomal location or next to a telomeric sequence. We conclude that the underlying recombination defect in ALT cells is restricted to telomeric sequences.

The alternative lengthening of telomeres pathway (ALT) is known to be one of two mechanisms by which immortal cells control telomere length.^{1,2} Although in the vast majority of tumors and cell lines telomerase is used to compensate for cell division associated telomere loss,³ ALT is also capable of telomere homeostasis. The ALT pathway is present in about 1/3 of all fibroblasts immortalized in vitro by SV40 Large T-antigen, while its occurrence in tumors has been estimated to be approximately 10% or less.⁴ Currently the diagnosis of ALT is based primarily upon exclusion of the telomerase pathway, which reflects our lack of understanding of the mechanism that engages the ALT pathway. The absence of telomerase activity as well as a very heterogeneous telomere length in immortal cells are two diagnostic markers of this pathway. In addition, the presence of nuclear structures called APB (ALT associated PML bodies) bodies has been considered to be specific for the presence of ALT.² Knowledge of the molecular changes in the ALT pathway would allow a more precise diagnosis. Important questions can be addressed once better diagnostic tools are available, e.g., are ALT cells often present in telomerase positive tumors, or is ALT a possible drug-resistance pathway for cancer cells in which telomerase has been inhibited for therapeutic purposes? The fact that ALT can also be found in vivo in certain types of cancer emphasizes the importance of understanding the mechanisms of this pathway of telomere length control.

Since there is good evidence that homologous recombination is the mechanism by which ALT cells maintain telomere length, we asked whether the underlying recombination defect in ALT cells is a general one which affects recombination between other homologous sequences as well. The experimental system utilizes an inducible recombination reporter system (pDR-GFP) stably transfected into cells, where the frequency of recombination is measured by the number of GFP expressing cells.⁵ The reporter system is composed of two non-functional GFP genes separated by a puromycin resistance gene (Fig. 1). The first GFP gene is interrupted by a unique 18 bp restriction enzyme site for I-Sce I, that includes a stop codon. The second GFP gene (incomplete GFP: iGFP) is a truncated version of GFP. The reporter system is induced by expressing the restriction enzyme I-Sce I, which causes a DNA double strand break (DSB) at the I-SceI target site within the reporter system. Following DSB induction the iGFP gene can serve as a donor for copying wild type GFP sequence into the former I-SceI site by a gene conversion event. Successful homologous recombination results in expression of a functional GFP gene that can be quantitated by FACS or fluorescence microscope. Some recombination products such as nonhomologous end joining (NHEJ) do not result in GFP expression.

We applied this system to two cell lines that were generated from T-antigen immortalized IMR-90 adult lung fibroblasts and hence share the same genetic background. The SW39 clone used the telomerase pathway while the SW26 clone used the ALT pathway to become immortal.⁶ Each cell line was stably transfected with the pDR-GFP reporter system, and clones with three or fewer copies of the transgene were analysed further (Fig. 1: Internal

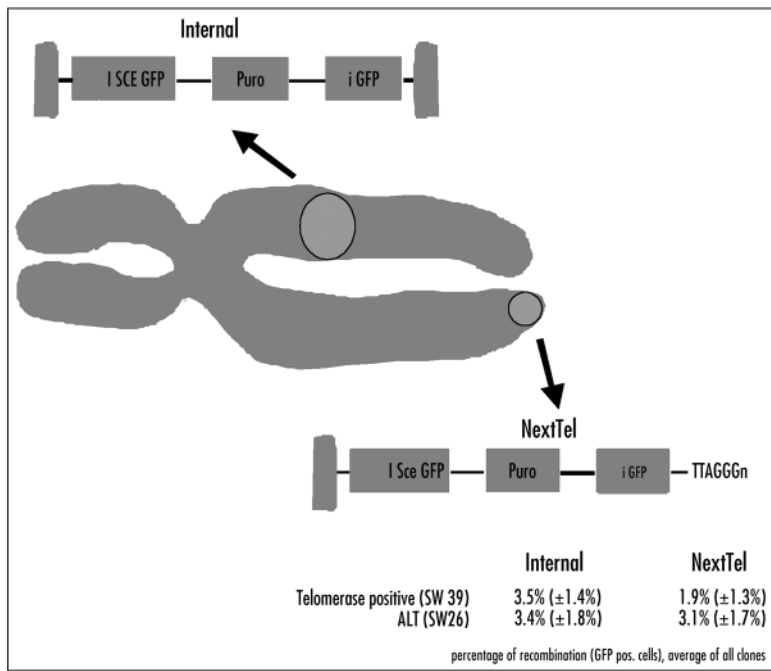


Figure 1. Measuring homologous recombination at various chromosomal positions. Transfection with the pDR-GFP reporter system yields random intra-chromosomal integration (Internal). Transfection with a chromosomal healing construct can lead to the seeding of a new telomere, thereby placing the reporter construct immediately adjacent to telomeric sequences (NextTel). The frequency of homologous recombination was identical in telomerase positive and ALT positive cells irrespective of the location of the reporter construct.

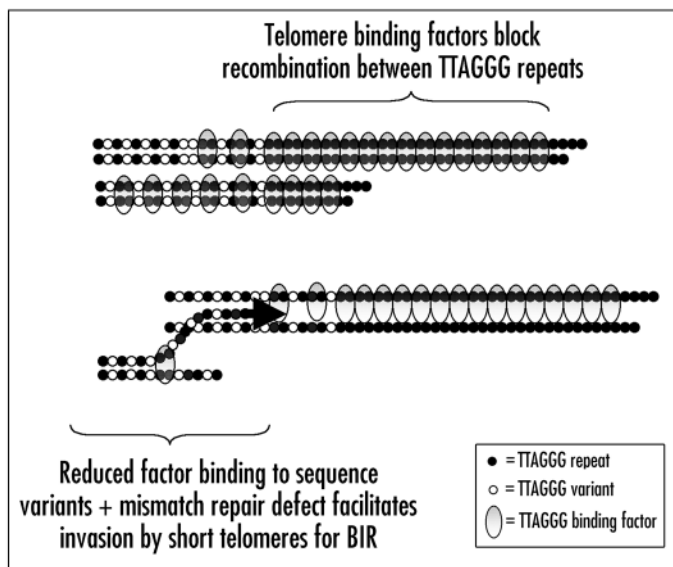


Figure 2. Break induced replication (BIR) model in the presence of a mismatch repair defect might lead to telomere elongation. TTAGGG sequence variants (e.g., TGAGGG) occurring at the base of a telomere, exhibit a reduced binding of telomeric factors that normally block recombination. Invasion of a critically short telomere into a longer donor telomere could lead to telomere elongation by non-reciprocal DNA transfer. Diverged sequences at the base of the telomere would result in nucleotide mismatches that would only be tolerated in the presence of a mismatch repair defect.

location). We found that the frequency of homologous recombination was the same in telomerase positive (average = 3.5%, 4 clones) and ALT positive (average = 3.4%, 5 clones) cells.⁷

In order to address whether adjacent telomeric sequences alter the frequency of homologous recombination, we modified our GFP reporter system by creating a chromosomal healing vector, that contains telomeric sequences at the end. Transfection with this linearized construct can cause chromosome breakage and seeding of a new telomeric end, so that the reporter system is positioned immediately adjacent to the newly formed telomere (Fig. 1: NextTel location).⁷ Homologous recombination experiments involving GFP sequences in sub-telomeric locations again showed identical frequencies of recombination in telomerase positive (average of 1.9%, 3 clones) and ALT positive (average of 3.1%, 4 clones) cells (only one copy of the transgene was present in each of these clones). This construct can also fail to break the chromosome and can remain as an intra-chromosomal insertion bearing 1.6 kb of telomeric sequences downstream of the reporter cassette followed by chromosomal sequences. Again, recombination experiments with clones having this type of insert showed no significant difference in the frequency of homologous recombination for telomerase positive compared to ALT positive clones (data not shown).⁷

Our data show that recombination involving homologous GFP sequences is identical in telomerase positive and ALT positive cells, irrespective of an intra-chromosomal or sub-telomeric location. We therefore conclude that the molecular defect in ALT cells is not affecting the general recombination apparatus but is likely to be telomere specific. The recombination data from sub-telomeric loci obtained with chromosomal healing constructs however should be interpreted cautiously. We do not find an altered frequency of recombination in sub-telomeric locations, which is in agreement with data obtained by Dunham et al.⁸ where no copy switching of a Neomycin marker gene was observed with sub-telomeric locations but where transfer to new telomeres was observed when the gene was located between blocks of telomeric repeats. However, chromosomal healing events create artificial sub-telomeric regions where a variety of normal sub-telomeric short repeats and telomeric sequence variants (e.g., TGAGGG) are not present. Furthermore, telomere seeding is the results of a selection process that allows only those healing events to occur where disruption of the normal sub-telomeric architecture at a particular chromosomal end is tolerated.

It has been shown previously that a sub-telomeric marker in *K. lactis* cells is rapidly dispersed to different chromosomes via recombination in the absence of telomerase activity.⁹ Recent data from Rizki et al¹⁰ indicate that in yeast cells lacking telomerase activity, cell survival is improved and ALT may be facilitated when a mismatch repair defect is present. Mismatch repair functions both to prevent mutations and to block recombination between similar but not perfectly homologous sequences (diverged sequences). If mismatch repair deficiency contributes to the defect in ALT cells, it is tempting to speculate that imperfectly homologous short repeats in the sub-telomeric regions or TTAGGG sequence variants at the base of the telomere might indeed become hot spots for recombination. One current model for telomere elongation in ALT cells involves break-induced replication (BIR), where a critically short telomere invades a longer donor telomere via a recombination process leading

to a nonreciprocal transfer of telomeric DNA onto the short end. The normal telomeric TTAGGG repeats become interspersed with TTAGGG sequence variants with increasing frequency at the base of the telomere.¹¹ Those noncanonical sequences could represent sites where telomere binding factors that inhibit recombination adhere less efficiently, increasing the possibility of recombination in cells containing mismatch repair defects.

We favour a model where telomeric sequences in telomerase positive cells are blocked from homologous recombination and this block is relieved in ALT cells (Fig. 2). In combining both models, critically short telomeric ends that contain few TTAGGG repeats still contain a substantial amount of TTAGGG sequence variants. The impaired docking of telomere binding factors to those sequence variants would partially relieve the recombination block at those short ends, which could potentially be elongated by recombination events. The presence of a mismatch repair defect would now allow recombination events to occur even in regions of diverged homology.

Our data suggests but does not prove that ALT cells have a defect in the regulation of recombination within TTAGGG or its sequence variants. A new reporter system will be required to be able to directly measure recombination occurring within these sequences. This would not only allow one to determine if ALT cells recombine between diverged TTAGGG sequences but also what degree of sequence diversity is tolerated. The identification of factors responsible for recombination in ALT cells would not only have an impact in ALT diagnostics but could also lead the way to more specific therapeutic approaches for certain types of human cancers.

SUMMARY

Our data show that in human cells general homologous recombination is not abnormal in ALT pathway cells. Identical frequencies of recombination are found in telomerase positive and ALT pathway cells at intra-chromosomal loci and loci in the immediate vicinity of telomeric sequences. We therefore conclude that ALT cells harbor a recombination defect that is restricted to telomeres and most likely involves TTAGGG and its sequence variants.

References

1. Greider CW, Blackburn EH. The telomere terminal transferase of Tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. *Cell* 1987; 51:887-98.
2. Henson JD, Neumann AA, Yeager TR, Reddel RR. Alternative lengthening of telomeres in mammalian cells. *Oncogene* 2002; 21:598-610.
3. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. *Eur J Cancer* 1997; 33:787-91.
4. Bryan TM, Englezou A, Gupta J, Bacchetti S, Reddel RR. Telomere elongation in immortal human cells without detectable telomerase activity. *Embo J* 1995; 14:4240-8.
5. Pierce AJ, Johnson RD, Thompson LH, Jasin M. XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. *Genes Dev* 1999; 13:2633-8.
6. Wright WE, Pereira-Smith OM, Shay JW. Reversible cellular senescence: implications for immortalization of normal human diploid fibroblasts. *Mol Cell Biol* 1989; 9:3088-92.
7. Bechter OE, Zou Y, Shay JW, Wright WE. Homologous recombination in human telomerase-positive and ALT cells occurs with the same frequency. *EMBO Rep* 2003; 4:1138-43.
8. Dunham MA, Neumann AA, Fasching CL, Reddel RR. Telomere maintenance by recombination in human cells. *Nat Genet* 2000; 26:447-50.
9. McEachern MJ, Blackburn EH. Cap-prevented recombination between terminal telomeric repeat arrays (telomere CPR) maintains telomeres in *Kluyveromyces lactis* lacking telomerase. *Genes Dev* 1996; 10:1822-34.
10. Rizki A, Lundblad V. Defects in mismatch repair promote telomerase-independent proliferation. *Nature* 2001; 411:713-6.
11. Allshire RC, Dempster M, Hastie ND. Human telomeres contain at least three types of G-rich repeat distributed nonrandomly. *Nucleic Acids Res* 1989; 17:4611-27.