# THE PLAN B OF TUMOR CELLS: AN INSIGHT INTO THE ALT MECHANISM

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## Introduction

Telomeres are the capping structures of chromosomes based on a repetitive sequence 5'-TTAGGG-3'. They protect chromosomes from external damage and procures their integrity. However, they present a really problematic handicap which is the inability of the replicative machinery to completely replicate telomeres, causing a progressive shortening of them with each round of cell division. When telomeres reach a limited length cell undergoes senescence, state in which is no more able to divide.
In order to avoid that, immortalized cells as stem cells or germ cells possess a mechanism to maintain telomere length making sure to keep telomere length stable. The rest of somatic cells do not possess the mechanism to maintain telomere length that ensures their unstoppable division. However, tumour cells go against these statement and reactivate a telomere lengthening mechanism, giving them the ability to proliferate without the problem telomere shortening.
The best known telomere maintenance mechanism (TMM) is the ribonucleoprotein enzyme telomerase which replenished replicative loss of telomeric DNA by using its integral RNA molecule as a template for reverse transcription of new telomeric DNA. Around the 85%-90% of tumors have an increased telomerase activity and the remaining 10%-15% maintain their telomeres length through an alternative mechanism referred to as ALT.
The ALT is a recombination-based telomere maintain mechanism which is more commonly found in mesenchymal origin and neuroepithelial origin cancers although the reason for this is not clear. It is thought this cells could control telomerase more tightly leading to a more facilitated activation of ALT. However, ALT has been also found in other cancer types, and intriguingly, it has also been showed to activate when tumor cells using telomerase are treated with telomerase inhibitors. Under this context, the interest for the study of ALT mechanism have increased in order
to understand this mechanism and lately to lead to a therapeutic approach.

In this review my aims have been:
- Offering a main view of the ALT mechanism.
- Describing the most important proteins implicated.
- Explaining the main features of cells using ALT.
- Putting ALT in a clinical context, discussing its prognostic value and the possibility of using some features for the detection and following of tumors using ALT.

**Prevalence of ALT in human cancer**

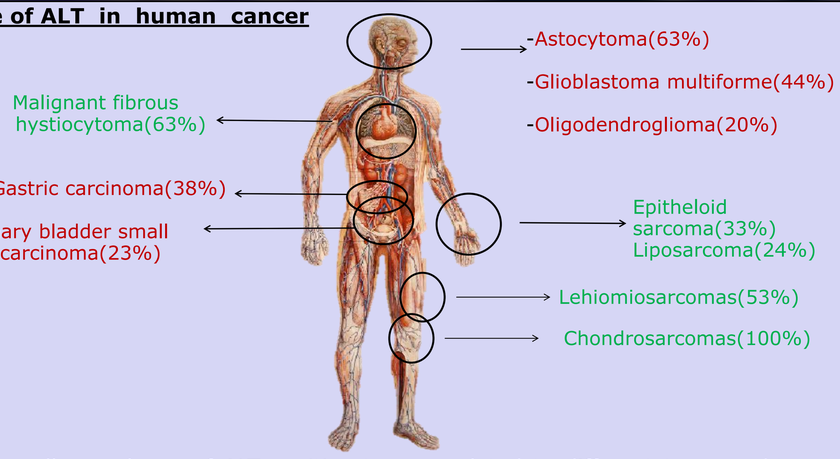



Figure 5: Overall prevalence of ALT positive tumors related to different tumor subtypes. The most prevalence ones are highlighted here. Sarcomas are indicated in green and carcinoma and central nervous system tumors are in red.
Modified from: http://e-ducativa.catedu.es/44700165/aula/archivos/repositorio/1000/1060/html/index.html

**ALT assays**

| ALT assay | Requirements | False positive | False negative |
|---|---|---|---|
| **Telomere length heterogeneity** **It remains the best established marker for ALT in human cells, including tumors archived under conditions where it is possible to extract high-quality genomic DNA.** | $1x10^4$ cells (TRF analysis; <1000 cells with FISH) Can be performed in frozen specimens | hTR and hTERT overexpression | ALT+ clone with catalytically dead hTERT |
| **ALT-associated PML bodies** **It is a well-established test for ALT. It is not affected by intra-tumoral heterogeneity observed in ALT because it assesses individual cells.** | Individual cells Especially useful for determining the ALT status of tumors as it can be used on paraffin embedded specimens | As the function of APBs in ALT+ cells and its relationship with it is not entirely clear, it would not be a utterly reliable test for ALT yet. | Some cell lines develop ALT mechanism without APBs. However, it has not been enough tested. |
| **Telomeric t-circle** **An increase in the level of t-circles has been used as a marker for ALT activity; however t-circles do not appear to be essential for, or confined to the ALT mechanism.** | $1x10^7$ in cell cultures, although it has not been tested in tumors. | Formed in response to DNA damage. Generated following depletion of Ku, or over-expression of mutant TRF2 in telomerase positive-cells. | T-circles can be formed independently of ALT in response to DNA damage. |
| **Telomeric SCE** **The value of T-SCE as a marker for ALT was demonstrated in ALT+ cells that lost other ALT markers but retained elevated T-SCE. Suggesting that T-SCE may be more tightly linked to the ALT mechanism than most other markers.** | Mitotic cells | Detected in short or damaged telomeres. Detected in a few cells using telomerase. | ALT+ line depleted for MUS81 had reduced T-SCE without any significant reduction in telomere length maintenance. |
| **C-circles** **The C-circle is the first candidate for an ALT specific molecule and an eligible target for a simple and versatile ALT assay.** | Detection by an isothermic polymerase based on RCA. | Not confirmed if C-circles are an integral part of ALT mechanism. | Not known |

Table 1: In here they are presented some phonotypical features from ALT tumors that could be used to assay ALT activity which would be useful in detection and following of ALT tumors. Table modified from: Henson JD et al. FEBS Lett. (2010). TRF (telomeric terminal restriction fragments), hTR (telomerase RNA subunit), hTERT (human telomerase reverse transcriptase), RCA (rolling circle amplification).

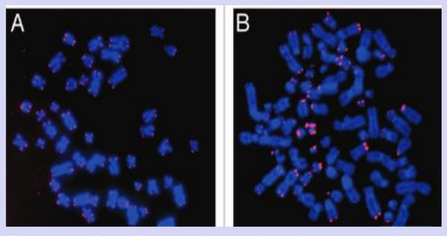



Figure 6: Example of telomere length heterogeneity assay. In A we have human lymphocytes ALT negative with homogeneous signal which reflects uniform telomere length. Conversely, in B we have chromosomes from a ALT positive cell line with wide variation in FISH signalling which reflects the large variation in telomere length. Source: Jegou T et al. Mol Bio Cell (2009).

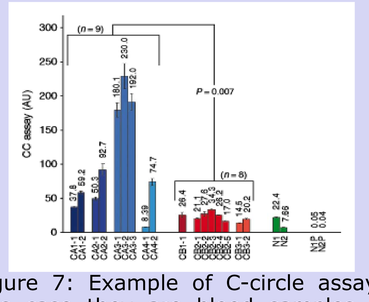



Figure 7: Example of C-circle assay. In this case they are blood samples from osteosacroma patients.
CA1-CA4 had ALT+ osteosarcomas whereas CB1-CB3 had ALT- osteosacromas. They also used controls, N1-2, N1P, N2P. It was observed C-circle increased in blood from ALT+ tumor patients. Source: Henson JD Nat Biotechnol (2009).

## Methodology

In order to carry out this review the methodology followed was based on three main statements:

- Papers taken from scientific data base like pubmed and google. Scholar
- First reading of reviews to get an approach into the topic and subsequent reading of experimental papers
- The search of papers was done under this keywords: **ALT, telomeres, cancer, recombination, APBs, proteins, phenotype.**

## Results

**ALT activation**

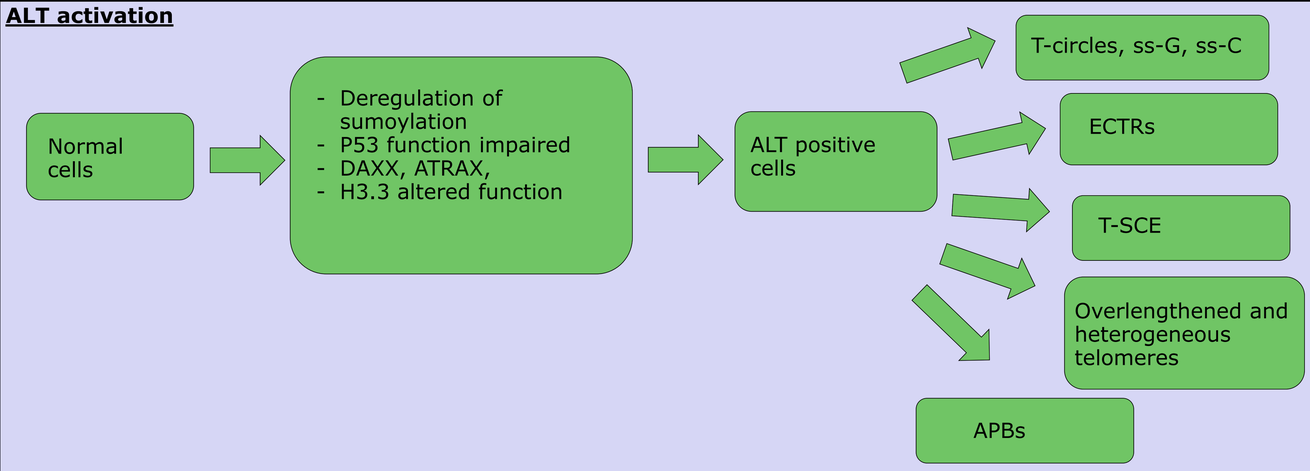



Figure 1: In same way some tumor cells reactivate telomerase, other tumor cells using ALT undergo mutation events, either genetic or genetic which affect c ells involved in DNA damage response, transcription regulation or sumoylation. That would lead to ALT activation, which is characterized for presenting the phenotypic features shown.

**ALT models**

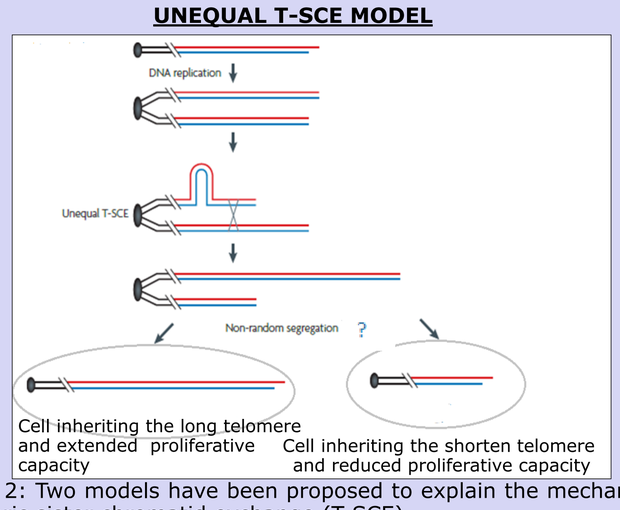



Figure 2: Two models have been proposed to explain the mechanism by which telomeres are elongated in ALT cells ; by an unequal telomeric sister chromatid exchange (T-SCE).
In the unequal T-SCE model, two sister chromatids exchange telomeric sequences unequally and when the cell divide one daughter will receive the lengthened telomere, acquiring extended proliferative capacity whereas the other cell will receive the shorten telomere resulting in a cell with reduced proliferative capacity.
In the homologous-dependent recombination DNA replication model, a chromatid from one chromosome invades telomeric sequence of another chromosome, using it as a template for the extension of its telomeric sequence. That leads to the extension of the second strand and eventually to a net gain of telomere length. Modified from: Cesare AJ et al. Nat Rev Genet (2010).

**Protein machinery and rol of APBs**

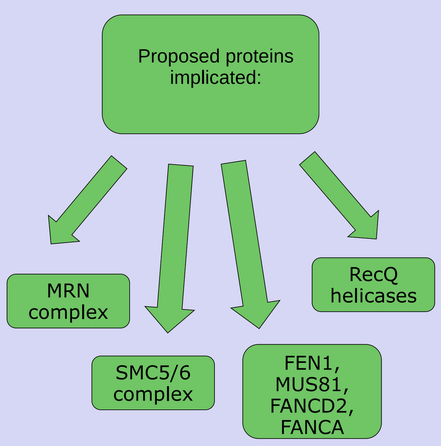
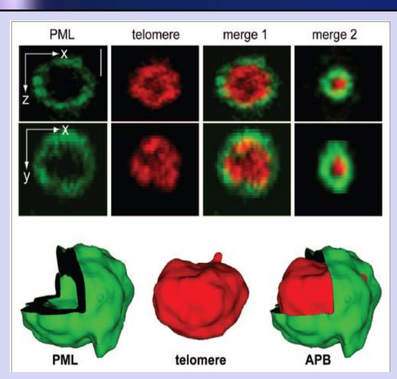



Figure 4: Many proteins have been identified as needed for ALT telomere elongation. They are proteins involved in recombination or proteins from DNA damage response system. And can function for instance as telomere lengthen enhancers or APB assembly promoters.



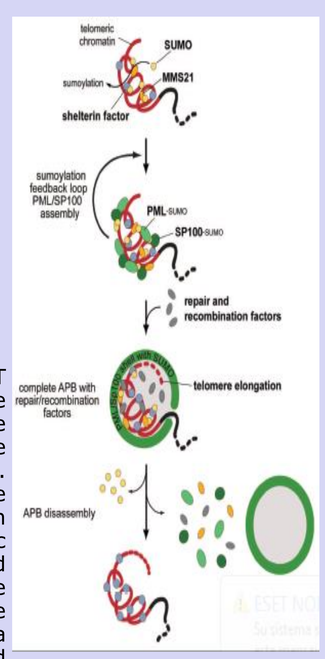




Figure 3 and 4: Some evidences had led to think that ALT associated PML bodies (APBs), would be the macromolecular platform where ALT activity would take place. In here it was detected signal the same place where telomeric DNA was found, co-localizing in the cell. According to this assumption, telomeric proteins would be sumoylated by SUMO complex triggering the accumulation of SP100 and PML, assembling the APBs where telomeric chromatin would be placed. At the same time, repair and recombination proteins like RecQ helicases would be recruited in order to allow telomere elongation. Once telomere elongation is done, APB would disassembly in a dynamic way. Source: Lang M et al. J Cell Sci. (2010) and Chung I et al Nucleus (2012).

## Conclusions

In this review we have seen that ALT mechanism is a recombination based TMM, even though the exact mechanism is not completely clear. Two models have been proposed, however, secondary mechanism could take place as well. Anyhow, these elongations are heterogeneous and telomeres are overlengthened, providing interesting features that along with other ALT phenotypic characteristics can be measured and could be useful to assay ALT presence. The proteins known to be required for ALT activity are present in normal cells, as they participate in recombination and repair functions. This has led to question whether if ALT might be or not a normal TMM, present in normal cells, in fact, this has been observed in vivo in epidermal stem cells in the absence of telomerase activity. Thus providing the fact that ALT mechanism might be a normal TMM, many questions emerge as, what is the reason of its existence in a normal cell and which mechanism uses a tumor cell to unregulated ALT normal mechanism.

Clinically, it has been observed that ALT mechanism dominates in some cancer subtypes, having a special preference for mesenchymal or neuropeithelial origin. However, it was intriguingly found that when tumor cells using telomerase were treated with telomerase inhibitors; an ALT+ phenotype appeared, suggesting an adaptive appearance of ALT in order to survive. This fact, make us think about the possibility of using, in future, a double therapy approach, in which telomerase and ALT inhibitors may be administrated in order to avoid this selection and surviving alternative. The major comprehension of ALT would let us choose the best targeting for ALT inhibitors.

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