Telomerase Data Regulación Cáncer/Patología

"It has now been clear that these mutations create a de novo binding site for ETS transcription factors"

"The block 6 repeats are predicted to hybridize along the distal portions of the TERT pre-mRNA. (a) Mfold analysis predicts that the block 6 repeats (marked by alternating black and grey to represent each 38 nucleotide repeat) hybridize along the distal portions of the TERTpre-mRNA to create an elongated structure that puts exon 6 and exon 9 closer together than exons 6 and 7, thereby potentially promoting minus beta splicing"

"Figure 1 | A distal block of repeats in intron 6 is necessary for TERT alternative splicing. ("

"Similarly, Li et al. introduced the C228T mutation into the TERT promoter in normal human bladder stem cells and this single event was sufficient to drive transformation of these stem cells ["

" CpG islands are usually clustered near the gene pro-moters where transcription initiation occurs. About 70% of the human gene promoters contain CpG islands, and there-fore DNA methylation has been thought to play an import-ant role in gene expression [128, 129 ]. Promoter DNA methylation has been recognized as one of the most fre-quent and stable ways of gene expression controlling mech-anisms."

"TP53/RB1 mutations and suggested that they might co-operatively contribute to the progression of bladder cancer."

"Clinically, tumors carrying TERTpMut frequently express higher levels of hTERT mRNA and telomerase activity compared with those having a wild type promoter highlighting the prognostic potential of TERTpMut and their potential use as a clinical biomarker ["

"Fig. 2 Mechanisms of hTERT regulation in cancer. Transcription factors and their binding sites, as well the positions of both hTERT promoter mutations, C228T and C250T, the hypermethylated region upstream to TSS (THOR) and TERT-miRNAs are shown. The cancer-specific mutations within the core promoter, at -124 and -146bp positions generate ETS binding motifs, leading to GABP transcription factor recruitment and consequently hTERT transcription. Binding of transcriptional activators (c-Myc) and repressors (WT1 and CTCF) to the hTERT promoter may be controlled by DNA methylation, in which methylated CpGs prevent their binding to the target sites, leading to hTERT activation (THOR region). MiRNAs targeting the 3â€™UTR promotes translation repression of hTERT. Black dots represent methylated CpG sites. "

"These results might support the hypotheses that TERTp-Mut are not the unique event responsible to initiate an oncogenic process explaining their presence in premalig-nant lesions and non-hTERT expressing tumors. TERTpMut usually occur in cancers with low rate of self-renewal, such as brain tumors, liver, melanocytes and even low-grade bladder cancers suggesting a role in triggering telomerase activation"

"Despitebothmutationsarefunctionally active the TERTpMut, C228T is significantly more frequent than the C250T "

". In cancer cells harboringTERTpMut,themu-tant promoter recruits GABPA and exhibits H3K4m2/3, an active chromatin mark. On the other hand, wild type cell lines exhibit the H3K27me3, a mark of epigenetic silencing, suggesting that only the mutant promoters are transcrip-tionally active"

"Functionally, hTERT promoter mutations are associated with the formation of consensus binding sequence (CCGGAA) at the E-twenty-six/ternary complex (ETS/ TCF) transcription factors (Fig. 2), providing a possible mechanism for cancer-specific upregulation of telomerase"

"E-twenty-six (ETS) transcription factor family, thus consti-tuting a novel mechanism of genetic activation in cancer and a possible driver genomic alteration"

"hTERT core promoter consists of 260 base pairs with multiple transcription-factors binding motifs that regulate gene transcription and telomerase activa-tion "

"two recurrent non-coding mutations within the hTERT promoter re-gion in both familial and sporadic melanomas [88, 89]. These two mutations were located at -124 and -146 bp upstream from ATG (chr5:1,295,228 G>A and 1,295,250 G>A, C>T on opposite strand). After the initial discov-ery, hTERT promoter mutations (TERTpMut) have been identified in multiple and distinct tumor types, such as glioblastoma, bladder and thyroid cancer, with different prevalence according to cancer type and histology"

"An unusual 1.1-kb region of 38-bp variable-number tandem repeats (VNTRs) that is conserved among Old World pri-mates was determined to be essential for the exclusion of exons 7 and 8 to produce minus beta splicing [36]. In a minigene context, the VNTR described may use RNA:RNA pairing as a mechanism to regulate splicing of hTERT [52]. The use of a VNTR that is far from the exon/intron junc-tions together with the potential use of RNA:RNA pairing as the mechanism to promote exon skipping makes the regulation of hTERT splicing atypical of those previously described,"

"In addition to transcriptional changes, hTERT splicing in cancer cells largely reverts back to the splicing pattern seen during development in which the pre-mRNA is often spliced into nonfunctional isoforms with only a smaller proportion of full-length transcripts that can be translated into functional reverse transcriptase."

"A working hypothesis to explain this observation is that the basal transcription machinery is unable to reduce transcription to the very low level that is optimal for telomerase function so the cell disposes of this excess transcription by alternatively splicing most tran-scripts into nonfunctional forms, leaving behind just enough protein to maintain telomere length in cancer cell"

"The need to fine-tune the regulation to produce â€˜just the right amountâ€™ of telome-rase may be because too little telomerase would not be enough to maintain telomere length, leading to increased genomic instability in cancer cells, but too much telome-rase may lead to runaway elongation of telomeres and result in adverse effects including inhibition of growth of the cancer cells."

"During fetal development, telomerase activity disap-pears before transcripts because of a dramatic shift in splicing pattern from full-length hTERT to mostly minus beta and other isoforms without reverse transcriptase activity [18]. This shift in hTERT splicing is a highly regulated process that in humans is both tissue and time dependent. hTERT was traditionally believed to be tran-scriptionally silenced in somatic cells post-development because the common method of examining hTERT splicing uses primers that examine the inner reverse transcriptase region of the gene, leading to the false assumption that no transcription of hTERT occurs in somatic cells after devel-opme"

"In addi-tion, two hTERT splice variants with intron retentions (INS3 and INS4) have been shown to be expressed primar-ily in telomerase-positive cells and act as dominant-nega-tive proteins that can bind DNA substrate but not the telomerase RNA component"

"Although minus beta has a premature stop codon and is therefore subject to nonsense-mediated decay, its transcripts have been shown to be translated into protein, and overexpression of the minus beta protein has been reported to confer a growth advantage on breast cancer cells"

"Figure 4. Telomerase (hTERT) is partially regulated by alternative splicing"

"Overexpression of the minus alpha tran-script inhibits telomerase activity in telomerase-positive cell lines, resulting in either cell death or senescence [42]. It is unknown whether the alteration of levels of the minus alpha splicing isoform is part of telomerase activity regu-lation in cancer cells to fine-tune telomere length."

"The 42-kb telomerase (hTERT) gene on human chromo-some 5p15.33 contains 16 exons and can be spliced into multiple isoforms [38]. To date, 22 isoforms of hTERT have been identified [39]. Besides the full-length transcript with all 16 exons, none of the identified alternative spliced forms has reverse transcriptase activity and they cannot elongate telomeres"

"Additionally, low-abundance transcripts have to compete with high-abundance transcripts for splicing machinery"

"Failure to recruit the necessary proteins for proper splicing is likely to be more detrimental to low-abundance transcripts due to their inherent low transcript levels. Hence, it is feasible that cells might have evolved more specialized regulatory mechanisms for low-abundance transcripts to ensure proper splicing"

"Although the coding sequence of hTERT is conserved among species, some of the intronic elements regulating hTERT splicing (discussed below) are only con-served among Old World primates [36]. In the mouse, mTERT is constitutively expressed in many tissues, whereas expression of hTERT in humans is more tightly regulated"

"However, studying the alternative splicing of hTERT poses a new challenge because previous studies of splicing have largely been based on highly expressed genes and the splicing of hTERT does not appear to conform to the established norm of alternative splicing regulation."

"Regardless of whether a cell has telomerase activity, almost all cells have an excess amount of hTR (hTERC), the telomerase RNA template [15]. By contrast, hTERT can be detected at relatively low levels in stem cells, progenitor cells, and even cancer cells"

"Telomerase is a ribonucleoprotein complex comprising a catalytic protein component with reverse transcriptase ac-tivity (hTERT) that uses a functional RNA component (hTR or hTERC) as a template to elongate telomeres"

"Initially, each human chro-mosome is capped by 15â€“20 kb of telomeric TTAGGG repeats"

"Alternative splicing affects approximately 95% of eukary-otic genes, greatly expanding the coding capacity of complex genomes."

"A model for the role of epigenetic modifications in telomere-length control. Normal-length telomeres have features of constitutive heterochromatin, such as subtelomeric DNA hypermethylation, hypermethylation of histone H3 at lysine 9 (H3K9) and histone H4 at lysine 20 (H4K20), hypoacetylation of histones H3 and H4, and heterochromatin protein HP1 binding at both telomeres and subtelomeres. This suggests that they have a compacted and â€˜closedâ€™ conformation, which is not accessible to telomerase and that represses recombination between telomeric repeats. As telomeres become shorter with increasing cell divisions, these heterochromatic marks are decreased from telomeres and subtelomeres, concomitant with increased histone actelylation. This leads to a more â€˜openâ€™ chromatin state, which allows a greater accessibility of telomere-elongating activities "

"However, mouse and human subtelomeric sequences can be methylated"

"Mammalian telomere repeats (TTAGGG) cannot be methylated because they lack CpG sequences, which are the substrates for mammalian DNA meth-yltransferases (DNMTs)"

"Telomeres consist of double-stranded G-rich repeats ending in a single-stranded 3 Ìoverhang (the G-strand overhang), which provides the substrate for telomerase2 (FIG. 1). The G-strand overhang can also fold back and invade the double-stranded region of telomeres, forming a protective structure known as the T-loop"

"Proper telomere functioning requires both a minimum length of TTAGGG repeats and the integrity of the shelterin complex"

"Table 1. Amino acid sequence identity between telomerase reverse transcriptases. Each value is % identity"

"It is also known that the telomeric shelterin complex must be removed before TZAP can access and cleave telomeric DNA"

"some CD4+ T cells had elongated telomeres and APCs had cor-respondingly shorter telomeres relative to pre-cellâ€“cell interactions"

" Similar to many other proliferative cell types, T cells enzymatically extend telomeres via telomerase, a DNA polymerase4. But this is not sufficient to prevent senescence during the massive antigen-driven proliferative expansion of T cells needed for pro-longed immunity5. "

"Eventually, a cellâ€™s telomeres become too short (less than 4 kb"

"Cellular senescence induced by DNA replication and telomere attrition contributes to organ dysfunction, inflammation and impaired immunity."

"We showed that telomere looping exists in long-telomere young fibroblasts and that telomere looping was reduced by in vitro aging. This is one possible explanation for why higher primates preserved the location of the TERT gene at the end of one of their chromosomes."

"(D) Simplified model of how TPE-OLD regulates hTERT expression in human cells during aging and cancer progression."

"While this result does not prove a causal role during cancer development, this series of experiments does demonstrate that telomere"

"shortening in cells that bypass replicative senescence leads to the hTERT locus entering into a more permissive state (e.g., increased hTERT mRNA expression) in the presence of oncogenic stresses, consistent with the disengagement of telomere looping"

"Thus, we tested if the knock-down of p21 would increase the expression of hTERT mRNAs and result in the inclusion of exons 7/8 in the short-telomere old BJ cells but not in the young BJ cells with long telomeres. We measured the expression level of hTERT transcripts in young and old BJ cells with and without p21 stable knockdown; mRNA containing exons 7/8 (exons coding for critical resi-dues in the reverse transcriptase domain of TERT) and exon 15/16 (most splice variants of hTERT contain exons 15 and 16), responsible for putative active hTERT and total hTERT variants respectively. Both the active and the total hTERT transcript variants significantly increased with the knockdown of p21 in old BJ but not in young BJ cells; however, we did not detect telomerase activity ("

"This indicates an intricate balance between chromatin modifications, methylation status, telomere length, and the expression of tissue-specific tran-scription and splicing factors that dictates the activation or repression of genes with replicative aging"

"We measured two histone marks associated with active chromatin H3K4 trimethylation (H3K4me3) and H3K9 acetylation (H3K4ac) and two histone marks associated with repressed chromatin H3K27 trimethylation (H3K27me3) and H3K9 trimethylation (H3K9me3), which have key roles in regulating gene expression [36]. We observed an increase in both H3K4me3 and H3K9ac across the TERT promoter in aged cells with short telomeres (Fig 3B). We also observed an increase in the repressive histone mark H3K27me3, but did not observe any sig-nificant differences in young or old BJ cells for the repressive histone mark H3K9me3. Collec-tively, this shows that the chromatin status of the hTERT promoter in old BJ cells with short telomeres is different and may be more transcriptionally permissive compared to young BJ cells with long telomeres"

"The relation-ship between DNA methylation and transcription in the hTERT promoter remains controver-sial in normal and cancer cells [31,32], but the transcription start site of hTERT retains little or no methylation in telomerase-active cancer cells for active transcription"

"(D) Percentage of adjacent allele (A) pairs versus separated allele (S) pairs was determined by 3D-FISH in normal BJ cells. BJ fibroblasts at different PDs were analyzed as indicated in the Materials and Methods. Cas9-mediated transient perturbation at the sub-telomeric 5p region was performed in BJ cells at PD25"

"(D) Higher primates also retain the location of the TERT gene at the end of their chromosomes. "

"We observed that hTERT is expressed at higher levels in two human fibro-blast strains with short telomeres compared to the same cells with long telomeres (Fig 1B, S1 Fig). As previously described, we did not detect any transcripts that contain the RT domain of hTERT (Fig 1B); thus, transcripts that could code for active telomerase were not observed."

"It is now known that hTERT transcripts can be detected in a variety of telomerase-negative cells and tissues, but the mRNA produced is not full-length mRNA capable of produc-ing active telomerase"

"transcripts containing only the RT domain of TERT (exons 5â€“10),"

"the TERT gene is only a megabase from the human chromosome 5p end"

" Therefore, aging and cancer are two ends of the same spectrum"

"he rapid lifespan increases have most likely put most humans out of balance with evolution. "

"When cells then enter crisis, in combina-tion with other genetic and epigenetic changes, instead of engaging senescence, cells develop genomic instability and an increased risk of cancer and activation of telomerase."

". One explanation is that humans historically died in child-birth, of accidents, infectious diseases, and/or starvation and have never had the evolutionary pressures to develop even bet-ter anticancer protection mechanisms. With the improvement in sanitation, the development of vaccines and antibiotics, safer working environments, and improved medicines and surgical procedures, humans have essentially doubled their average lifespan in the last 150 years"

"Finally, if one deletes Tert or Terc (encoding the functional RNA template component of telomerase) from inbred strains of mice ( 81 ), telomeres do progressively shorten and, in later generations, mice develop aging phe-notypes (stem cell dysfunction, cardiomyopathies, insulin resistance, diminished stress responses, and only a modest increase in cancer) similar to humans ( "

"n average human weighs about 60 to 80 kg and lives about 75 to 80 years compared with inbred strains of mice that weigh about 20 to 25 grams and live approximately 2 to 3 years. Yet humans and mice get about the same incidence of cancer. For this to make sense, humans would have to be at least 100,000 times more resistant to cancer compared with mice ( 53"

" In contrast, the bowhead whale, which lives almost 200 years and is believed to be the longest-living mammal, has âˆ¼ 1,000 times more cells compared with humans. Similar to elephants, whales are rarely found to develop cancer. The bowhead whale genome was also recently sequenced, and the investigators proposed that increased copies or variants in DNA-damage repair genes (mutations in ERCC1 and PCNA and FEN1 duplications) may account for cancer-free longevity in whales ( 79 ). "

" This paradigm has led to the concept of evolutionary tradeoffs. Cancer resist-ance due to repressed telomerase and short telomeres might limit regenerative capacity, thus increasing the likelihood of age-dependent degenerative diseases, particularly as animals get older and their telomeres undergo further shortening."

" It is well established that most large mammals also have more cells and generally longer lifespans that require more cell replications, which theoretically should increase the mutational burden and augment cancer risk. However, Peto pointed out that cancer risk does not always scale with size ( 72 ). Large, long-living mammals show no increase in cancer risk compared with small, short-lived ones. Known as Petoâ€™s paradox ("

"However, recent studies have shown that in the general population individuals with inherently long telomeres are also at a higher risk for major cancers ( 67â€“71 ). How do we explain this apparent paradox? "

". This is consistent with the observation that almost 70% of all cancers are in the 65-and-older segment of the population."

". It is known that telomerase is active during early human fetal development, then becomes silenced in most tissues at approximately 3 to 4 months gestation ( 62 ). Thus, when telomeres reach a certain initial length ( âˆ¼ 15â€“20 kb) during human development, three-dimensional chromatin structures involving telomere position effects over long distances (TPE-OLD; refs. 63, 64 ) may silence the TERT gene. As part of cancer progression, as telomeres shorten, the chromatin-silencing effects may become relaxed, resulting in a permissive environ-ment for telomerase promoter mutations and telomerase reactivation "

"These include mutations/deletions in the TERT promoter ( 36â€“44 ), engagement of TERT alternative splicing ( 58, 59 ), TERT gene amplifi cation ( 60 ), and epigenetic changes ( 44 ). Another possibility is that the human TERT gene may autoregulate itself because it is located very close to the telomere end of chromosome 5 ("

"). It was reported that mutant TERT promoters exhibit the H3K4me2/3 mark of active chromatin and recruit the GABPA/ B1 transcription factor, although the wild-type TERT allele retains the H3K27me3 mark of epigenetic silencing and does not recruit the GABPA/B1 transcription factor. "

" Indeed, almost all malignant tumors have very short telom-eres. One could speculate that if telomerase was expressed at very high levels, then telomeres might elongate greatly and this could have detrimental consequences. Alternatively, if telomerase was not activated suffi ciently, then telomeres would continue to shorten with continuing cell divisions, and the cells would eventually stop dividing. Thus, there is unlikely to be a selective advantage to having more than suffi cient telomerase to work on a very small number of the shortest telomeres."

" Although it is believed that these mutations activate telomerase activity (by converting conserved regions within the TERT promoter to an ETS transcription factor binding site) "

". Somatic mutations in the proximal promoter of the human telomerase reverse tran-scriptase gene (TERT ) are now considered the most common noncoding mutation in cancer. For example, the vast majority of primary melanomas (67%â€“85%), glioblastomas (28%â€“84%), liposarcomas (74%â€“79%), and urothelial cancers (47%) contain TERT promoter mutations"

" In human cells, the bypass or escape from senescence can be experimentally demonstrated by abrogating important cell-cycle checkpoint genes [such as TP53 (p53), CDKN1A (p21), CDKN2A (p16INK4a), and RB1 (pRb)], leading to increased numbers of cell divisions of potentially initiated premalignant cells ( 31â€“34 ). Eventually, cells enter a state termed â€œcrisis,â€ which is a period where cell divi-sion and death are in balance. In crisis, due to chromosome end fusions, there are chromosome breakageâ€“fusionâ€“bridge events, leading to genomic instability, rearrangements of chromosomes, and eventually activation or upregulation of telomerase and progression to malignant cancers ( "

"It is a common misconception that normal senescent cells undergo apoptosis and die. It is now recog-nized that senescent cells can secrete factors that can infl u-ence age-associated diseases "

" The shelterin complex protects chromosomes from end-to-end fusions and degradation by forming special t-loopâ€“like structures ( 24 ), thus masking the very ends of chromosomes from being recognized as double-strand DNA breaks. "

"Telomeres appear to lose ~50 bp per cell doubling in vitro, but why do they lose much less in vivo? Is the end replication problem really rate limiting for what most scientists call replicative senescence, or is it a result of stressful culture conditions that accelerate telomere shortening or that induce premature growth arrest? "

"Thus, the spatiotemporal expression of telomerase is tightly regulated in human cells and may have evolved to prevent the early onset of age- related diseases, whereas the detrimental effects of this regulatory setup would generally occur in post- reproductive years and hence would not have strong and lasting evolutionary implications."

"However, with continued cell divisions and progressive telomere shortening, the TERT gene becomes permissive for transcriptional activation and may be detrimental to the organismâ€™s fitness post- reproduction late in life."

" One mechanism to explain this process would involve telomere looping that changes the chromatin patterns near the TERT and CLPTM1L loci at an early age and silences TERT expression; however, with ageing and progressive telomere shortening, TERT could become permissive for reactivation, increasing the risk of cancer. The concept of antagonistic pleiotropy163â€“166 was proposed to help explain evolutionary theories of ageing applied to long- lived organisms"

"antagonistic pleiotropy?. Telomerase is detected in the early stages of human development (for example, in the blastocyst stage) but becomes silent in a tissue- specific manner during fetal development in all somatic tissues and remains silenced in most tissues unless cancer occurs."

"he telomere position effect (TPE) is another mechanism generally associated with transcriptional repression of genes close to telomeres, as previously demonstrated in both yeast and humans"

" genetic anticipation in future generations"

"T cells102, thus demonstrating that some highly proliferative normal cells could express regulated telomerase activity"

"some cancer cells that lacked telomerase underwent spontaneous remissio"

"Even though human TERC was known to be present in telomerase- silent cells, it was surprising that the introduction of human TERT was sufficient to reconstitute"

"so there is concern that effective telomerase inhibitors might engage this ALT- based survival pathway"

"However, so far, there have not been any anti- telomerase therapies approved for any indication. This was certainly not from lack of trying but most likely a result of the long lag period from inhibiting telomerase until telomeres were short enough to cause cells to enter crisis and undergo apoptosis"

"As telomerase activity was absent in most normal tissue, and almost all human tumours (85â€“90%) not only constitutively expressed telomerase but also had short telomeres, the inhibition of telomerase became, and remains, an attractive target for cancer therapeutics"

"The human TERC (also known as TR or hT R for human telomerase RNA) gene was cloned in 1995 (reF.28) and is ubiquitously expressed in all normal human cells"

"Telomerase activity was subsequently found in a variety of species, which all generated their speciesâ€™ characteristic telomere repeat sequence in an RNA- dependent manner,"

" Finally, the single- stranded telomere overhang loops back and invades the double- stranded telomeric repeats to form a t- loop, so there are no exposed free ends that may trigger DNA damage responses. This further helps to preserve genomic integrity and provide end protectio"

"same sequence was found to be conserved among more than 90 eukaryotic species60 including all mammals6"

"The ends of telomeres are protected not only through invasion of the terminal single- stranded DNA overhang into duplex TTAGGG repeats to form a t- loop so that there is no free end but also through binding of a complex of proteins (termed the shelterin complex) that protects the ends of telomeres to prevent the linear ends of chromosomes from being recognized as DNA damage needing repair. "

"â€˜telomeresâ€™, as did Haldane and Darlington, from the Greek words for â€˜endâ€™ (telos) and â€˜partâ€™ (meros)"

"provides a new understanding of why human telomeres are fairly similar in length at birth and how progressive telomere shortening can change cell physiology and affect diseases associated with ageing before becoming terminally short and without inducing a DNA damage signal"

"In addition, we discuss the observation that the introduction of the cDNA for TERT into telomerase- silent human cells is often but not always sufficient to produce cell immortalization"

"TERT alternative splicing may also be involved in silencing telomerase activity during development, thus limiting the maximal length of human telomeres18,19, but what regulates alternative splicing of TERT is largely unknown. In addition to alternative splicing, other mechanisms such as epigenetic changes20 involving telomere 3D looping21â€“25 may occur."

"During early human development, telomerase is active but becomes transcriptionally silenced between 12 weeks and 18 weeks of gestation"

"The ALT pathway occurs in only ~10â€“15% of cancers14, whereas telomerase activation occurs in 85â€“90% of all human cancers"