
Telomeres and Brain Tumors

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1. Introduction

Brain tumors are a large and heterogeneous group of neoplasms affecting the central nervous system that, despite the progress of the modern medicine, still represent a great challenge for physicians all over the world. The annual global age-standardized incidence of primary malignant brain tumors is ~3.7 per 100,000 for males and 2.6 per 100,000 for females. Rates appear to be higher in more developed countries (males 5.8, and females 4.1 per 100,000) than in less developed countries (males 3.0 and females 2.1 per 100,000). Conversely, the incidence of both primary malignant and non-malignant brain tumors is about 10 - 14 cases per 100,000/year all over the world, with white males having the highest rate. Males also generally have higher rates of primary malignant brain tumors while females have higher rates of non-malignant tumors, primarily meningiomas. Worldwide age-standardized mortality for primary malignant brain tumors is ~2.8 for male and 2.0 for females per 100,000. Mortality rates differ significantly by histology and age. For example, glioblastoma multiforme (GBM) has a 5-year survival rate of 3.3%, low grade gliomas, such as pilocytic astrocytomas, oligodendrogliomas, and ependymomas have 5-year survival rates of over 70%, while anaplastic astrocytoma, malignant gliomas and lymphomas have 5-year survival rates less than 40% [1].

Due to these dramatic epidemiological data, in the past decades we assisted to the birth of an intensifying interest in understanding the causes of brain tumors among the scientific community. Despite notable advances achieved during recent years in both surgical and chemo/radiotherapeutic approaches, an improved survival has not been clearly documented, especially for primary malignant brain tumors. This is partially due to the tumors' intrinsic clinical and molecular heterogeneity. As a matter of fact, choice of initial treatment, prediction of survival, stratification of patients, prediction and monitoring of response to therapy, still represent some

of the greatest challenges in the management of patients affected by brain tumors. In these settings, different studies have been performed to better understand the pathophysiology of cerebral neoplasms, with the aim to identify new molecular prognostic markers and therapeutic targets. However, until now, molecular biomarkers that effectively predict response to therapy and/or survival outcomes in brain tumors are limited. Consequently, there is a strong need to develop novel and independent markers of prognosis for these neoplasms. One of the cellular structure candidate to accomplish this role is represented by telomeres.

Telomeres consist of long tandem arrays of TTAGGG repeats, bound by proteins, placed at the end of linear chromosomes, which are involved in several essential biological functions [2, 3]. These non-coding telomeric repeats represent a buffer zone preventing the adjacent coding region of the genome from erosion. In normal human cells, the telomeres decrease by some 5-20 repeats with every cell division [4]. Therefore telomere shortening limits the number of times a cell can divide.[5] Hence, they can be considered the mitotic clock by which cells count their divisions and regulate the onset of replicative senescence in somatic cells [6-8]. The alteration of telomere length homeostasis affects telomere structure and leads to genomic instability by generating chromosome end-to end fusion and chromosomal abnormalities [9]. It has been demonstrated that telomeres shortening could initiate successive events, such as aberrant fusions or recombination of the end of chromosomes, genomic instability, loss of cell growth control, and finally cancer development [10, 11]. Hence, cells exhibiting critical telomeres shortening and genomic instability present an increased capacity of dicentric chromosomes formation and susceptibility to oncogenic transformation [5, 12, 13]. Several studies showed the presence of an hypervariability of telomere length in different human solid tumors, including brain tumors, suggesting for telomeres and proteins involved in the modulation of their length a possible clinical role as diagnostic/prognostic marker or therapeutic target for cancer treatment.

2. Telomeres

Human telomeres are regions of 4-15 kilobases of repetitive hexameric (TTAGGG) $_n$ guanine-rich DNA sequences at the ends of each chromosome [14]. They end with a single-stranded 3'-overhang that folds back and invades its complementary strand to form a T-loop [15]. Formation of this structure is presumed to involve the insertion of the 3'-telomeric overhang into duplex telomeric DNA, its hybridization with the cytosine-rich strand, and dislodgment of the guanine-rich strand into a displacement loop (D-loop) [16]. It has been demonstrated that T-loops are especially well-adapted to shield the ends of chromosomes from DNA repair and DNA damage-sensing mechanisms [15, 17]. Moreover, a complex of telomere-specific proteins, named shelterin complex, binds and caps telomeres, further preventing chromosomal ends from being recognized as DNA double strand breaks (DSB) by the DNA damage response (DDR) machinery [18, 19]. As the lagging strands of telomeres are incapable of being fully replicated during each round of cell division, telomeres undergo progressive shortening during normal cellular proliferation. Eventually, they become so short that they trigger the DDR, causing cell crisis [4, 18, 20]. This normally results in replicative senescence and even-

tually checkpoint-driven cell death and apoptosis, defining cellular lifespan and safeguarding an organism against unlimited cellular proliferation and cancer [19, 21, 22].

Shelterin complex proteins interact selectively with telomeric DNA and localize to telomeres. In its most abundant form, the complex is composed of six core components: TRF1, TRF2, POT1, TIN2, TPP1 and RAP1[16]. TRF1 and TRF2 (Telomeric Repeat Factors 1 and 2) recognize duplex telomeric DNA whereas POT1 (Protection of Telomere 1) associates with the single-strand telomeric DNA present at the 3'-overhang [23, 24]. The scaffolding subunit TIN2 (TRF1-interacting nuclear protein 2) binds simultaneously to TRF1, TRF2 and TPP1, whereas TPP1 (previously known as TINT 1 [25]) connects POT1 with TIN2 [25-31]. A sixth component, RAP1 (Repressor Activator Protein 1), is a TRF2-associated factor [32]. Via protein–protein interactions, these DNA binding factors bring a number of other proteins to telomeres, where they localize to form a variety of complexes [33, 34], playing a key role in telomere capping and length regulation (see Figure 1).

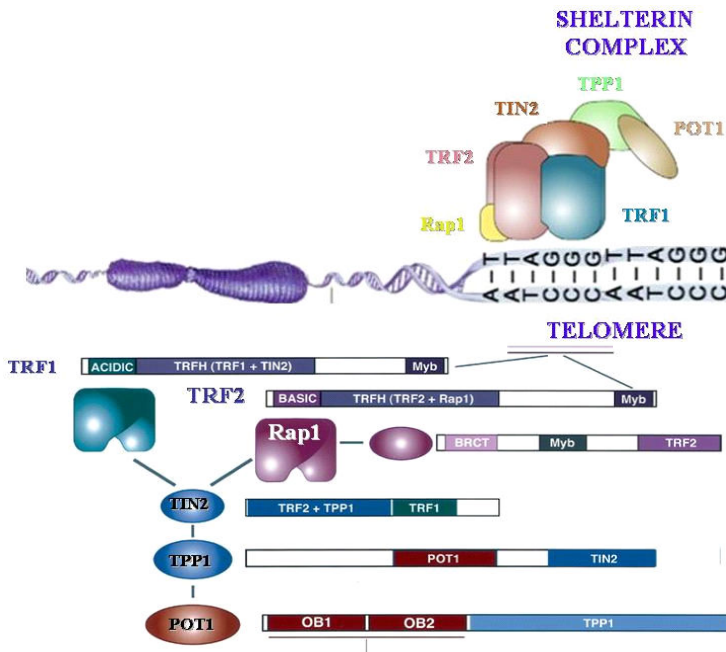


Figure 1. Schematic representation of the six subunits of the Shelterin complex on the telomeric DNA and of their molecular structure, including domains, protein interactions and DNA binding sites.

In addition to this 6-member complex, Shelterin may also be present in the form of sub-complexes that lack either TRF1 or TRF2/RAP1[30, 34-41]. While the importance of these different sub-complexes remains unclear, the particular function of each of the three DNA binding subunits (TRF1, TRF2 and POT1) is well-understood.

TRF1 has a key role in the modulation of telomere length. Its ADP-ribosylation, allow telomerase to bind telomeres and start their elongation.

TRF2 serves to block recognition of telomeres as double-strand DNA breaks. Loss of TRF2 function leads to the activation of the ATM kinase (Ataxia telangiectasia mutated kinase), formation of telomere dysfunction-induced foci (TIFs), and induction of either senescence or apoptosis [21, 36, 42-45]. Moreover, in cells lacking functional checkpoints, telomeres devoid of TRF2 serve as substrates for the NHEJ (non-homologous end-joining) repair mechanism and give rise to interchromosomal fusions [41, 46]. TRF2 also appears to protect telomeres, at least in part, through its ability to promote T-loop formation and maintenance [15, 17].

POT1 serves to hide the 3'-telomeric overhang from telomerase and from DNA damage sensing mechanisms. Hence, the loss of POT1 leads to the activation of the ATR kinase (ATM and Rad3-related kinase), formation of TIFs, and induction of either apoptosis or cell cycle arrest [38, 42, 47, 48]. In cells that lack functional checkpoints, POT1 dysfunction leads to a loss of telomerase regulation that results in longer telomeres [31, 39].

Therefore, through the interaction with the shelterin complex proteins, telomeres protect chromosomes from recombination, end-to-end fusion, and recognition as damaged DNA, providing a means for complete replication of chromosomes.

In summary, telomeres serve as a molecular clock that controls the replicative capacity of human cells and their entry into senescence, but they also contribute to the functional organization of chromosomes within the nucleus and participate in the regulation of gene expression [49, 50].

2.1. Telomere length modulation

During a process of DNA synthesis and cell division, telomeres shorten as a result of the incomplete replication of linear chromosomes. This progressive shortening represent the so-called 'end-replication problem'.

As previously described, in order to prevent degradation by exonucleases or processing as damaged DNA, the telomere 3' single-strand overhang folds back into the D-loop of duplex telomeric DNA to form a protective 'T-loop', which is reinforced with TRF2 and other telomeric DNA-binding proteins that constitute the shelterin complex [51]. These proteins have a fundamental role in the modulation of telomere length, allowing telomeres elongation or conversely promoting their attrition.

In human cells, several pathways regulating telomeres length have been identified.

The most important mechanism is represented by telomerase, a highly specialized ribonuclear reverse transcriptase enzyme that catalyzes extension of 5'-ends of the lagging DNA strand by adding TTAGGG repeats onto the telomeres using its intrinsic RNA as a template for reverse transcription [52]. Two major subunits of the human telomerase core complex have been identified, named h-TERC and h-TERT. The former serves as a template for telomeres elongation; instead, the latter subunit (h-TERT) contains a reverse transcriptase domain that catalyzes this reaction [53] (see Figure 2).

TELOMERE LENGTH MODULATION

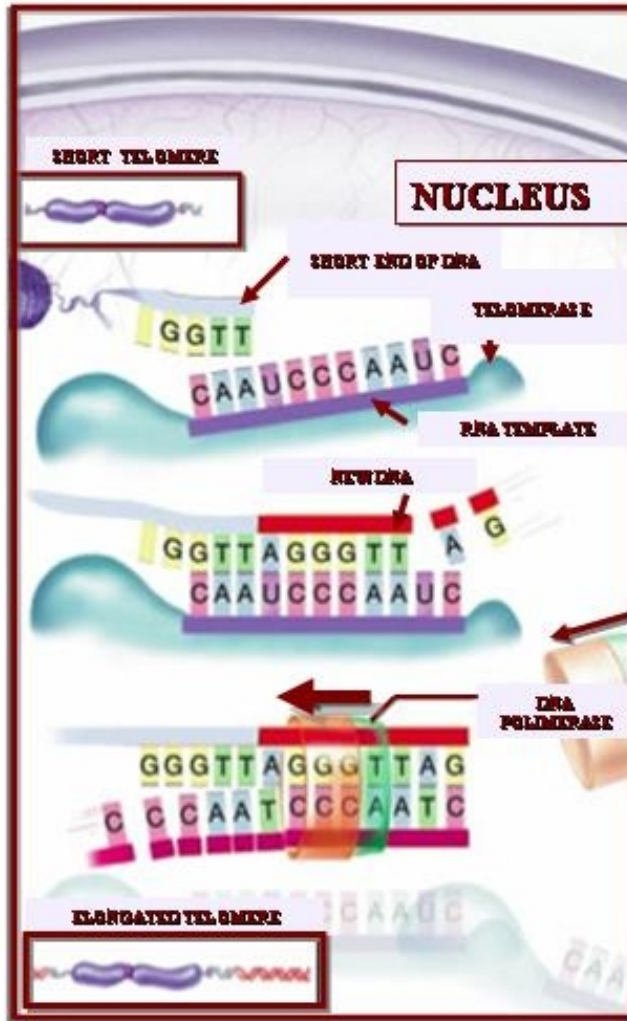


Figure 2. Schematic representation of telomere elongation by telomerase

However, the length and structure of telomeres are also controlled by a variety of proteins, working exclusively at the telomere or contemporarily participating in DNA repair process. Collectively, these telomeric proteins may function to protect telomere integrity and function, to connect the DNA damage/repair network with the controls of cellular senescence, to monitor telomere homeostasis and modify the access of telomerase to telomeres. Two major proteins are the TRF1 and TRF 2 that are localized at telomeres [54]. These proteins play key roles in

the maintenance of telomere function and structure modifying telomerase activity [41, 55, 56]. Moreover, a recent evidence shows that TRF1 interacts with other telomere-binding molecules and is integrated into the functional telomere structure [57]. TRF1 accepts adenosine diphosphate (ADP)-ribosylation catalyzed by the tankyrase-poli-ADP-ribose polymerase (TANKS-PARP) complex. The ADP-ribosylation of TRF1 reduces its ability to bind telomeric DNA, allowing telomerase to elongate telomeres and extending the cellular life span [58-60].

In most somatic cells telomerase is produced at very low levels. In contrast, many malignant cells are able to upregulate this enzyme and extend their survival through continuous telomeric elongation [61].

The vast majority of tumor cells use telomerase as preferred mechanism for telomere maintenance, whereas only 10-15% of all cancer lose the functional activity of telomerase and use the telomerase-independent Alternative Lengthening of Telomeres pathway (ALT) that operates via DNA repair and recombination processes [22].

ALT was first deduced in human cell lines from the fact that some telomerase-deficient lines were able to be maintained in culture for many hundreds of population doubling times [62]. Later phenotypic studies revealed that, unlike telomerase-positive cells, ALT-dependent cells almost always contain heterogeneous telomere length distribution and form ALT-associated promyelocytic leukemia (PML) bodies or APBs. [63, 64] These phenotypes are either undetectable or have very low levels of activity in normal somatic cells. [22]

Several characteristic features are associated with ALT activity [65]. First, telomere length distribution is highly heterogeneous and ranges from less than 3 kb to more than 50 kb [66]. In contrast, in human telomerase-positive cells all telomeres typically have a similar length of around 10 kb. Second, ALT-positive cells have been described to contain several classes of extrachromosomal telomeric repeats (ECTR) in the nucleus. ECTRs comprise double- and single-stranded circular molecules, linear telomeric DNA, and t-complex molecules that consist of high molecular weight DNA with highly branched structures [67-70]. These DNA fragments might be products of t-loop resolution by recombination enzymes. The function of ECTRs in the telomere lengthening process is unknown but the amount of partially single-stranded telomeric (CCCTAA)_n DNA circles (C-circles) appears to correlate with ALT activity [71]. Third, the occurrence of telomere sister chromatid exchanges (T-SCE) is generally increased in ALT cells [72, 73]. Fourth, in ALT cells promyelocytic leukemia nuclear bodies (PML-NBs) associate with some telomeres [64, 74]. These complexes are called ALT-associated PML-NBs (APBs) [64, 65].

Which type of telomere maintenance mechanism is active seems to depend on the origin of the tumor. ALT is rarely found in carcinomas but frequently activated in tumors of mesenchymal and neuroepithelial origin like osteosarcomas, liposarcomas or astrocytomas [75, 76].

Therefore, at least two mechanisms of telomere maintenance, telomerase activity and the recombination-based ALT, may be more or less prevalent in different tissues undergoing tumour formation, leading to the aforementioned features [77, 78]. The activation of such a mechanisms causes the alteration of telomere length that is reflected by a hypervariability of telomere length already observed in different human solid tumors [79-81], and also in brain

tumors [82-87]. Hence, analysis of telomere length variations and of length modulation mechanisms may provide promising information for their potential role as prognostic marker or therapeutic target in cancer.

3. Telomere in aging and disease

Aging can be defined as the progressive decline of tissue function that eventually results in mortality [88]. Aging is a natural occurring process and not a disease state. While aging is inevitable for all humans, the speed of age-related functional deterioration varies considerably amongst individuals. Why some individuals reach frailty earlier than others is not understood and genetic factors as well as environmental exposures are believed to modulate the senescence process. Currently, aging is viewed as a generalized process occurring in all organ systems in a parallel fashion [88]. However, recent studies point towards a much higher degree of complexity in the aging process and accumulating data support the notion that diseases incurred during life may accelerate the aging of certain organ systems [89].

Telomeres shorten as we age. Consequently, telomere length has been postulated as a marker of "genetic age" (mitotic clock), as a fundamental explanation for the aging process, and has been marketed as a simple predictor of longevity. Telomere length indeed reflects the cell's past proliferative history and future propensity to apoptosis, senescence, and transformation. However, cellular aging is not equivalent to organ or organismal aging. Studies in humans have attempted to relate short telomeres to longevity. In a provocative initial publication from the University of Utah, individuals around 60 years of age who had the longest telomeres lived longer than did subjects with the shortest telomeres, but the most associated cause of death in the latter group was, inexplicably, infection, and those with shorter telomeres did not have a higher rate of cancer deaths [90]. Heart disease as the cause of death was also more common in subjects with the shortest telomeres. Subsequent studies have produced conflicting findings. The Cardiovascular Health Study of subjects over 65 years of age found that individuals in the shortest quartile for telomere length were 60% more likely to die than those in the longest quartile [91]. Causes of death related to short telomeres were infectious. Two twin studies at older age also correlated shorter telomeres with poorer survival [92, 93]. Finally, a cohort study that looked at participants at time zero and after 10 years found that death within 10 years was significantly more common in those with shorter telomeres [94]. In contrast, these associations have not been confirmed in other studies of older subjects. Njajou and colleagues reported that telomere length failed to predict survival, but correlated with years of healthy life [95]. In a Danish study of people aged 73 to 101 years, telomeres correlated with life expectancy in simple univariate analysis but, when corrected for age, did not predict longevity [96]. In Dutch men with a mean age of 78 years, telomere length eroded with aging but failed to correlate with mortality [97]. In a Finnish investigation, telomere length did not predict overall mortality [98]. Finally, in an analysis from California, short telomere length predicted death from cardiovascular disease in women but not in men, where the rate of shortening predicted mortality rather than length itself [99]. Collectively, data on association between telomere length and aging are

quite inhomogeneous and contradictory, whereas data on the role of telomere length in human disease are more consistent.

Telomere dysfunction and telomere length alterations have been reported as responsible of various human pathologies, ranging from neurodegenerative to inherited genetic diseases. In the last decade, several studies elucidated telomere's role in the pathophysiology of different diseases at a molecular level. For this reason, measuring of telomeres in peripheral mononuclear cells or in whole blood samples has been applied to patients with a multitude of disease states [100].

Dyskeratosis congenital (DC), is a rare inherited bone marrow failure disease, and can be considered the classic "telomere disease". The inherited defect in X-linked dyskeratosis congenital was identified in a gene named *DKC1* [101]. *DKC1* encodes dyskerin, a protein that binds to the RNA component of telomerase and stabilizes the telomerase complex. Inheritance is X-linked recessive, autosomal dominant, or autosomal recessive. In addition to the diagnostic triad of nail dystrophy, lacey reticular pigmentation, and oral leukoplakia, patients with DC are at very high risk of bone marrow failure (BMF), cancer, pulmonary and liver disease, and multiple other medical problems [102, 103]. Several studies demonstrated that patients with dyskeratosis congenita have accelerated telomere shortening and that telomere length measurement by flow fluorescence *in situ* hybridization in peripheral blood lymphocytes is an important diagnostic test for DC: in particular, age-adjusted values provide a quantitative measure of disease severity, with shortest telomeres associated to severe variants of DC [104, 105].

Much attention has been given to individuals with atherosclerotic disease and, as a common rule, telomeric length of peripheral mononuclear cells seems to be a strong predictor for disease progression. In particular, leukocyte telomere length is associated with measures of subclinical atherosclerosis [106] and with HDL cholesterol levels [107]. Moreover, cellular aging reflected by shorter leukocyte telomere length is a predictor for advanced atherosclerosis and cardiovascular disease risk [108]. Patients with myocardial infarction have shorter telomeres as compared to controls [109], and reduced leukocyte telomere length has been linked to increased coronary artery calcium [110] and reduction in left ventricular mass.[111] Shortened telomeres have been observed also in white blood cells of patients affected by diabetes type I and II,[112-114] neurodegenerative disease including dementia and Alzheimer disease [115-119], ischemic stroke [120]. All these results suggest an important role of telomere and of its length modulation mechanisms in the pathophysiology of several common diseases, often age-related, encouraging new researches to improve the knowledge about telomere biology and to translate these new informations in the clinical practice for a better patient's management in terms of diagnosis, treatment and prognostic stratification.

4. Telomeres and cancer

Functional telomeres protect chromosome ends from recombination and fusion, and are therefore essential for maintenance of chromosomal stability [3, 121, 122]. The alteration of telomere length homeostasis affects telomere structure and leads to genomic instability by

generating chromosome end-to-end fusion and chromosomal abnormalities [9]. It has been demonstrated that telomeres shortening could initiate successive events, such as aberrant fusion or recombination of the end of chromosomes, genomic instability, loss of cell growth control, and finally cancer development [10, 11]. Cells exhibiting critical telomeres shortening and genomic instability thus present an increased capacity of dicentric chromosomes formation and susceptibility to oncogenic transformation [5, 12, 13].

The phenomenon of telomeres alteration during carcinogenesis and cancer progression is well known and established at the molecular level [123-125]. Several studies demonstrated the presence of telomere length alterations in different human solid tumors, such as prostate, breast, colorectal, head and neck cancer [81]. These alterations are widely variable, resulting in both telomere attrition and elongation as compared to adjacent normal tissues. In the majority of cases, telomere length seems to be reduced in human tumors [126-131], even if it tends to increase along with malignancy of tumors [79, 132, 133].

In particular, telomere dysfunction has been extensively studied in the most common solid tumors. These studies demonstrated telomere length modification in different histologies as compared to normal tissue due to the alteration of telomere length modulation mechanisms [81]. This differences have been widely analysed in order to identify new cancer biomarkers, to investigate the eventual correlation with patient's prognosis and to discover new targeted molecular therapies.

In breast cancer, multiple studies have showed that telomere shortening is associated with increased risk and poor outcome, and can thus be used as a prognostic factor to predict the course of disease. The first report describing the use of telomere length as a possible prognostic marker in breast cancer was published by Odagiri and colleagues [130]. In this study, telomere length analysis was performed in a cohort of 41 patients diagnosed with breast cancer and showed that telomere length was significantly reduced in tumor tissues (8.1 ± 0.6 kb) as compared to that of the adjacent normal breast tissues (9.7 ± 0.5 kb) in 18 of 22 patients ($p > 0.05$). Subsequent studies confirmed the potential role of telomere length as clinical biomarker in breast cancer, including Fordyce et al. [127] who reported measurements of telomere length in 2 independent sets of breast tumors containing a total of 140 samples. This study showed that telomere alterations include both telomere attrition and elongation. In fact, only 50% of all tumors had telomere values in the normal range. Moreover, in this study telomere length was associated with tumor size ($p = 0.02$), TNM stage ($p = 0.004$), 5-year overall survival ($p = 0.0001$) and 5-year disease-free survival ($p = 0.0004$) and, in particular, reduced telomere length was associated with nodal involvement ($p < 0.0001$).

Recent investigations concerning prostate cancer suggest that reduced telomere length is associated with poor clinical outcome and markers of disease progression [126, 128]. The first reported study was performed by Donaldson and colleagues in 1999 and represented a retrospective investigation of the relationship between clinical outcomes in patients with organ-confined prostate adenocarcinoma and telomere length [128]. In this archival case-controlled study, association analysis for telomere length and survival and telomere length and biochemical recurrence indicated by PSA levels of > 2.5 ng/ml, revealed that reduced

telomere length correlated with death ($p < 0.0001$) and disease recurrence ($p < 0.0001$), even if there was no statistical association with patients' ages at diagnosis, nodal statuses, pathological grades or Gleason sum scores. This finding was recently confirmed in a larger retrospective population-based study comprising 77 men who underwent prostatectomy between 1982 and 1995 [126]. In this cohort, telomere length was a predictor of time to recurrence when adjusted for age at diagnosis, Gleason sum score and pelvic node involvement.

Although relatively rare, researches on the role of telomeres in lung cancer showed that telomerase expression and activity can be considered important contributors to the malignant phenotype in lung epithelial cells, and have been proposed to be of potential prognostic value [134]. Conversely, reports on the use of telomere length to predict lung cancer progression are scarce. Shirotani et al. investigated the relationship between telomere length and various characteristics of tumor cells in 46 lung cancer specimens, comprising 40 primary and 6 metastatic lesions [135]. In partial accordance with recent studies in breast and prostate cancers [126, 127], the authors observed both elongation (2 cases) and reduction of telomere length (13 cases) in the 16 small cell carcinomas of the sample set. The 2 cases with telomere elongation were associated with a poor prognosis. Similarly, in the adenocarcinoma samples of this study, both telomere reduction and elongation were observed, but a clear association with patients prognosis was not reported. Hirashima et al. evaluated the prognostic significance of telomere length alterations in cancer and normal lung tissues obtained from 72 patients with histologically confirmed pathological stage I-IIIa non-small cell lung cancer (NSCLC) [136]. In this study, the 25 patients (34.7%) with alterations in telomere length, elongation or attrition, had significantly shorter survival durations than those of the others.

The use of telomeres as prognostic factors in colorectal carcinoma has been suggested in several studies performed by the groups led by Siewert and coworkers [132, 137] and by Iniesta and colleagues [133]. It is interesting that these studies showed an opposite relationship as compared to studies in other cancer types [126-129, 131], with longer telomeres associated with poor prognosis. This difference may be explained considering the different regulation of telomerase expression in colorectal epithelial cells. Normal colorectal epithelium, in fact, has been showed to contain cells of possible stem cell origin that are telomerase-positive and presumably counteract telomere attrition due to physiologically high cell proliferation rates, and total cell loss due to physiological shedding in this specialized cell compartment [138]. Consequently, it is possible that the existing high telomerase activity may affect cells undergoing tumor initiation and results in elongated telomeres in cells of colorectal cancer.

Studies indicating the prognostic potential of telomeres have also been reported for head and neck cancer. Patel et al. studied telomere alterations in tumor and adjacent normal tissues in 110 patients with head and neck cancer (squamous cell carcinomas of the oral cavity, larynx and pharynx) and 40 patients with precancerous and benign conditions (leukoplakia, submucous fibrosis, erythroplakia, hemangioma) [79]. Telomere lengths in this sample set were significantly lower in malignant tissues as compared with the tumor adjacent normal tissues. In addition, 2-year disease-free survival analysis showed that patients with longer telomeres in malignant tissues had poor disease-free survival. These findings are in agreement with that reported for colorectal carcinoma and lung [132, 133, 135, 137], and in contrast to other cancer

histologies [126-129]. A possible explanation for these interesting discrepancies in head and neck cancer is the fact that telomerase activity was observed in over half of the adjacent normal tissues. As before discussed for colorectal carcinoma, altered regulation of telomerase expression in cells undergoing transformation may explain the elongated telomeres in the tumors.

Collectively, these findings suggest that hypervariability of telomere length in various human solid tumors probably reflects the differential regulation of telomerase expression in cancer cells and depends on the different stages of carcinogenesis and tumor progression. Therefore, analysis of telomere length may have a clinical relevance as a prognostic marker in human solid tumors, whereas analysis of the mechanisms of length modulation, such as telomerase and ALT, can suggest new molecular target for advanced therapeutic strategies.

5. Telomeres and brain tumors

Brain tumors research is being performed worldwide at a remarkable pace, with some of the more recent promising studies focused on identification of aberrant genetic events and signalling pathways, tumor stem cell identification and characterization, modulation of tumor immunological responses, combination therapies, and understanding of the rare long-term survivors. Identification of additional indicators will enable better patients' stratification and individualization of treatment is needed to more accurately determine patient's prognosis and to identify novel therapeutic approaches that can optimize patient's outcome. A growing body of knowledge suggests a potential role of telomere length measurement in different tumors. Nevertheless, even if its clinical use is not completely established, a number of studies demonstrated that it can be helpful to patients stratification, to provide useful information about patient's prognosis and, in some case, to suggest new therapeutic strategies in cancer.

Although not entirely consistent in the type of telomere alteration, i.e., attrition vs. elongation, and unclear on the underlying mechanisms, multiple studies have showed that telomere dysfunctions are associated with parameters of clinical outcome in patients with brain tumors. A possible explanation for these interesting discrepancies in brain tumors is the fact that different expression and/or altered regulation of telomerase expression in tumor cells may reflect the underlying biology of telomere maintenance and its dysfunction over time. In telomerase positive tumor cells, telomere length is balanced by telomere shortening due to cell division and telomere elongation by telomerase. Therefore, telomere length is maintained by telomerase activity that can be influenced in different ways and by various factors. This mechanism keeps tumour cells proliferating and growing by the stabilization of their telomeres which is essential to maintain the unlimited dividing potential and to escape from 'crisis' [49, 123, 139]. As well as in almost all malignant tumors, World Health Organization (WHO) high grade brain tumors are associated to higher telomerase activity than benign tumors, such as schwannomas, meningiomas [140] or normal brain tissue [141]. Increased telomerase expression has been also associated with higher proliferative index, tumor grading, age, vascular and endothelial proliferation [142], poor outcome [83, 143, 144], and it increases with malignancy from low-grade to high-grade brain tumors [83, 145].

Therefore, understanding the context and mechanisms by which telomeres length contribute to cancer development is the next logical research step and may represent an interesting research field in order to elucidate brain tumors biology. Moving toward the study of molecular mechanisms controlling telomere length, including telomerase and ALT, will not only provide insight into the complex etiology of brain tumors but also promises to provide novel targets for cancer therapy.

In these settings, several studies have been performed to analyze telomere biology in brain tumors, with the aim to better understand the pathophysiology of these tumors for improving prognosis of patients affected by such terrible neoplasms.

5.1. Astrocytic gliomas

Astrocytic tumors represent about the 23% of primary brain and CNS tumours and, in particular, astrocytomas and glioblastomas (GBMs) account for 76% of all gliomas. The incidence of such tumours tends to increase with age, although some histological variants are more frequent in specific age ranges. For example, pilocytic astrocytomas affect exclusively childhood, being the most common brain tumor between 5 and 14 years old. Conversely, the incidence of glioblastomas increases with age, with the highest rates in the 75 to 84 years old. The prognosis can be different according to histology. For low grade astrocytomas (grade I and II), prognosis is usually good and a gross total surgical excision alone represents a sufficient therapeutic strategy. Conversely, for high grade astrocytomas (i.e. glioblastomas WHO IV), although a multimodal therapeutic strategy consisting of radical surgical removal, chemotherapy and radiotherapy, prognosis remains poor. For example, five-year survival rates are 94% for pilocytic astrocytomas but are less than 5% for glioblastomas. Survival generally decreases with older age at diagnosis. Children and young adults have better survival for most histologies [146].

Several studies focused on astrocytomas showed an association between telomerase activity and reduced telomeres length in high grade tumors. Liu et al. analyzed telomere length in a series of astrocytic tumors. Telomere length measurement was performed using a telomere specific restriction enzyme and Southern blot analysis. The authors observed a progressive shortening of the telomere restriction fragments (TRFs) in astrocytomas from WHO grade I to IV as compared to TRFs of normal brain tissue, with no significant differences between primary and recurrent GBMs [87]. These findings support the hypothesis that telomere shortening is one of the important genetic events during transformation of astrocytomas and progression to higher grades.

Subsequent studies were focused on the analysis of the relationship between telomere length and telomerase activity, suggesting new interesting hypothesis about telomere maintenance mechanisms during the neoplastic transformation and progression of astrocytic tumors. In 1997, Morii and colleagues, analysed telomere length and telomerase activity in a series of 20 gliomas (WHO grade I to IV), including 1 pilocytic astrocytomas, 7 oligoastrocytomas, 1 anaplastic astrocytoma (AA) and 11 GBMs. In their series, telomerase-positive samples had a mean TRF length of <10 kb, whereas telomerase-negative samples all had long heterogeneous TRFs which exhibited an increased signal peak from 10 to 20 kb. The authors concluded that

telomerase-negative gliomas had longer TRFs compared with telomerase positive ones, suggesting that, in addition to the telomerase-dependent mechanism, an alternative telomerase-independent mechanism (ALT) for telomere maintenance may be present in human gliomas [86]. Hiraga et al, in 1998, performed the most complete analysis of telomere length in human brain tumors, comparing TRFs length and telomerase activity in 160 neuroepithelial and non-neuroepithelial brain tumors. Among grade I-IV astrocytomas, the authors compared telomere length between telomerase-positive and telomerase-negative samples vs normal brain tissue (NBT). TRFs were shorter in tumors with telomerase activity than telomerase-negative samples and NBT samples. Specifically, the detection rates of telomerase activity were widely different for different histopathological entities. Telomerase activity was detected in none of pilocytic astrocytomas, in 20.0% (3 of 15) of grade II astrocytomas, 40% (6 of 15) of anaplastic astrocytomas and 72.3% (34 of 47) of glioblastomas, suggesting that telomerase activity tend to increase with the malignancy of astrocytic tumors. In particular, all pilocytic astrocytomas (3 of 3) were telomerase negative and showed longer TRFs as compared to normal brain tissue. TRFs in grade II astrocytomas and anaplastic astrocytomas with telomerase activity were shorter as compared to the same tumors without telomerase activity. Lastly, 80% of the progression GBMs exhibited reduced mean TRF length (7.747 kb) compared with NBT and origin tumors, and telomerase was reactivated in 100% (10 of 10) of cases. In summary, the mean TRF length of tumors with telomerase activity was significantly shorter than that of tumors with undetectable telomerase activity for each tumor entity [139].

These results suggest that telomerase activity strongly correlates with malignancy and potential progression of the astrocytic tumors, being often associated with reduced telomere length.

These findings were confirmed by Le et colleagues, in 1998 [83]. The authors performed an analysis of telomere length and telomerase activity in a series of 69 grade I-IV gliomas. From the analysis of all series, the authors concluded that telomerase activity is present in most glioma samples (72%), but that the frequency of such activity increases with malignancy, being higher in high grade gliomas (HGGs) than in low grade gliomas (LGGs), and is often associated with telomeres shortening. This can be explained with the relative low replicative rate of LGG tumor cells. When the proliferative activity increases, such as during the progression to HGGs, the increased mitotic activity causes a progressive telomeres shortening. In this condition, the activation of telomerase activity represents the only way to escape from cell crisis and apoptosis. This can explain the presence of high telomerase activity and reduced TRFs only in a small percentage of LGGs that probably presents a more aggressive behavior than the telomerase negative LGGs with normal or elongated telomeres. This demonstrates an increasing telomerase activity with the more malignancy of gliomas samples, underlying the role of telomerase and TRFs length as marker of malignancy.

Similar findings were found by Maes and colleagues in 2007 [145]. They conducted a study focusing on the relationship between telomerase activity and telomere length in a series of 53 intracranial tumors, including 2 low grade astrocytomas, 1 anaplastic astrocytomas, and 11 GBMs. Again, in this study the authors demonstrated that telomere length was reduced in high-grade tumours, whereas it was compatible or elongated as compared to normal brain

tissues in low-grade astrocytomas, suggesting that telomerase activity with shortened telomeres correlates with the aggressive growth of high-grade gliomas.

Harada and colleagues [147] analyzed possible differences of telomerase activity and telomere length among primary and secondary GBMs. Summarizing their findings, TRFs were always shorter than NBT samples, but not statistically significant differences between primary and secondary GBMs were found. Interestingly, although the latter presented significantly higher levels of telomerase activity and hTERT expression than the former, telomere length was anyway shorter than normal brain tissue. The authors explained this apparent discrepancy suggesting that telomerase activation occurs late in carcinogenesis, when the high replication rate of tumor cells already caused telomeres shortening. At this point, activation of telomerase represents the principal mechanism to escape from apoptosis and cell death. Conversely, in primary GBMs, shorter telomere length can be explained by a reduced telomerase activity that might have less influence on carcinogenesis and, hence, other unknown factors might facilitate their cellular immortality.

Although the vast majority of the researches have been performed focusing their attention to telomere length and telomerase expression in astrocytic gliomas, recently several studies showed an increasing interest also to the analysis of the relation between ALT mechanism, telomere length and patients prognosis.

The presence of elongated telomeres and of an alternative mechanism of telomere length maintenance (ALT) was demonstrated to be associated to a better prognosis in patients affected by GBMs by Hakin-Smith et al. in 2003 [148]. Their work represents the first report describing the relationship among ALT pattern, telomere length, and prognosis in human GBMs. In this study, the authors analyzed telomerase activity and telomere lengths in 77 GBM patients. ALT phenotype patients had a median survival of 542 days compared with 247 days in those without the ALT phenotype. Moreover, ALT phenotype was associated with elongated telomeres, benign biology and better prognosis. Therefore, the presence of ALT could be a positive prognostic marker in glioblastoma multiforme.

The first study that analyzed the ALT prevalence among different grades of astrocytic tumors was performed by Henson and coworkers in 2005. In their study, the authors analyzed the prevalence of ALT phenotype and telomerase activity in a series of 40 astrocytomas, composed by 7 WHO grade II-III astrocytomas and 33 GBMs. This study showed for the first time that the prevalence of ALT associated to elongated telomeres is significantly higher in grade II to III astrocytomas as compared to GBMs.

On the basis of Henson's results, in 2010 Slatter and colleagues conducted a study focusing on the analysis of ALT prevalence in a series of 48 astrocytic tumors from grade I to IV. In agreement with previous studies by Hakin-Smith and Henson [148, 149], ALT phenotype was more frequent in low grade astrocytic tumors, whereas telomerase activity was prevalent in the higher grades.

Collectively, these studies demonstrated that telomerase activity correlates with malignancy of astrocytic tumors, being higher with the increasing WHO grading [83, 86, 87, 139, 145, 147, 150]. Therefore, telomerase activity and reduced telomeres should be considered a potential bio-

marker of aggressive behaviour of these neoplasms. Conversely, low grade astrocytomas are usually telomerase-negative and have compatible or elongated telomeres as compared to normal brain tissue [83, 86, 139, 145, 149]. In these tumors, the only telomere length maintenance mechanism is represented by ALT [149, 150]. Henson et al [149] and Slatter and colleagues [150] documented that ALT phenotype is associated to elongated telomeres and it is more frequent in low grade astrocytic tumors as compared to GBMs. Moreover, Hakin-Smith and colleagues [148] demonstrated that, despite the ALT phenotype associated to elongated telomeres is rarely documented in GBMs, when present it is associated to a longer survival, suggesting its possible role as a positive prognostic markers in GBMs. These findings can be explained considering the biological role of telomeres. In normal tissues as in neoplastic cells, telomeres undergo progressive shortening during each cell replication. When telomeres are critically shortened, the cell cannot preserve anymore its own chromosomic ends and goes toward cell crisis and subsequent apoptosis. Therefore, telomeres represent the biologic clock of cells and constitute one of the most important structure that can preserve cells from senescence. This is the reason why tumor cells need a robust telomere maintenance mechanism to allow an high replicative activity, escaping from cell death. It is well known that high grade brain tumors are malignant neoplasms with an high replicative activity. Consequently, they need a mechanism of telomere maintenance that allows cell division avoiding excessive telomere shortening that can determine cell death. Telomerase seems to accomplish this role with success, especially in high grade tumors. This can be the explanation of the higher telomerase activity found in brain tumors with the increasing of WHO grading. Hence, the highest levels of telomerase expression are found in GBMs. Conversely, low grade astrocytic tumor have a slow replication rate and thus they does not need telomerase. According to the aforementioned studies, in these tumors the slow growing rate is accompanied by a sluggish cell division rate. This can be the reason why the most part of the studies in the literature documents elongated telomeres in low grade tumors. In these neoplasms, the main telomere length maintenance mechanism is represented by ALT. Being specific of benign or slightly aggressive tumors, ALT and elongated telomere are associated to a better survival [149, 150] even if these features are found in a low percentage of high grade brain tumors, such as GBMs [148]. When low grade tumors progress to higher grade, the increasing replicative activity causes a progressive telomere shortening. In this condition, the only way to escape from cell death is the activation of telomerase activity. This is typically observed in progression GBMs [139].

In summary, high telomerase activity and reduced telomere seem to be features of high grade astrocytic tumors, whereas elongated telomeres and ALT phenotype are specific of low grade ones and, therefore, correlates with a better prognosis.

Different studies have also been performed to analyze the role of the Shelterin proteins in the pathophysiology of several neoplasms, including brain tumors. Telomere-specific DNA-binding proteins, such as TRF1, have been put forward as additional candidates for the role of molecules modifying telomerase activity, and they have been suggested to play key roles in the maintenance of telomere function [41, 55, 56]. It has been demonstrated that overexpression of TRF1 inhibits telomere elongation in telomerase-positive cells [151], resulting in gradual and progressive telomeres shortening to the "mortality stages," the proliferative

barriers that lead to a non-dividing state and cell death [24, 151, 152]. The mutation or deletion of TRF1 can result in telomere elongation and extend cell survival [151]. Overexpression of a dominant negative TRF1, which removes endogenous TRF1 from telomeres, results in telomere lengthening in telomerase-positive cells [151] [153]. Therefore, the expression of TRF1 is considered a physiological homeostatic mechanism that controls the proliferative potential of normal cells by inhibiting the activity of telomerase [151].

It has been demonstrated that TRF1 is expressed in astroglial brain tumors of different grades, whereas it is not expressed in normal brain tissue. Such expression decreases from low-grade through high grade astrocytomas [123]. This finding may suggest that the loss of TRF1 expression capability, being the result of down-regulation of TRF1 expression in malignant gliomas cells, may play a role in the cell immortalization of astroglial brain tumors.

Furthermore, according to the role of telomerase in the elongation of telomeres in high grade astrocytic tumors, several studies suggested its possible role as molecular target for new therapeutic strategies. Marian et al demonstrated that the telomerase antagonist Imetelstat efficiently targets glioblastoma tumor-initiating cells leading to a decreased proliferation and tumor growth [154]. Gurung and colleagues showed that Thymoquinone induces telomere attrition, DNA damage, cell cycle arrest and apoptosis in the glioblastoma cells by inhibiting the activity of telomerase [155]. Lin et al documented that Butylideneophthalide (BP) inhibits proliferation and induces senescence in human glioblastomas by downregulating hTERT expression and consequently telomerase activity [156]. From this perspective, telomerase represent a promising target for new specific therapeutic approaches for the personalized treatment of telomerase-positive high grade astrocytic tumors.

5.2. Ependymomas, oligodendrogliomas and mixed tumors

Ependymomas are tumors that arise from ependymal cells lining the cerebral ventricles and the central canal of the spinal cord. They represent the 5-6% of intracranial gliomas (69% occur in children) and 60% of spinal cord gliomas (96% occur in adult) [157]. In pediatrics they are usually intracranial (the most common localization is the posterior fossa), whereas in adults they tend to be spinal. The treatment of choice is the radical surgical excision followed by radiotherapy (XRT), whereas the role of chemotherapy is very limited. The operative mortality is 5-8%, the 5 year survival is 20-30% in pediatrics and 80% in adults [158, 159].

Data on telomeric alterations in adult and pediatric intracranial ependymomas are slightly inhomogeneous. In 2008 Ridely et al. performed an analysis of telomere length in a series of 21 primary and recurrent pediatric intracranial ependimomas from 7 patients (6 primary tumors and 15 recurrences) [84]. Telomerase activity was detected in 19 tumors (86%), and particularly it was evident in 11 of 14 primary tumors and in all recurrent tumors. Mean telomere length ranged from 7.3 to 16.7 kb, with telomere maintenance observed in five of seven patients (71%). Of these five cases, four showed telomere lengthening and one had compatible TRFs in relapsed tumors as compared to the primary tumor. Conversely, telomere shortening occurred in two of seven recurrent cases (29%).

The authors concluded that variable TRFs length and evidence for telomerase-mediated telomere maintenance were present in the majority of pediatric ependymoma recurrent cases (71%), whereas TRFs shortening was evident in a minority (29%). These results implicate telomerase-mediated telomere maintenance as a key mechanism facilitating tumor progression in pediatric ependymoma.

This was demonstrated by the fact that all recurrent tumors analyzed were telomerase positive, although telomere length was reduced only in small percentage of cases.

In the same year, Tabori et al evaluated TRFs length in a series of 26 pediatric intracranial ependymomas (grade II and III), analyzing an eventual correlation with patients' prognosis [85]. Telomerase activity was detected in 73% of tumor samples but TRFs length was widely variable (mean 6.5 kb, range 3.6–9.1 kb). Based on these findings, although ependymomas rely predominantly on telomerase activity to maintain their telomere length, the authors concluded that there was no correlation between telomere length and telomerase expression or survival. Although it seems to emerge that the majority of intracranial ependymomas (especially in children) shows telomerase activity, telomere length appears to be widely variable. Therefore, there is a lack of a firm correlation of telomere length with telomerase expression that do not allows to get definitive conclusions about their possible role as prognostic marker in these neoplasms. It is more probable that these findings reflect an high molecular variability of intracranial ependymomas.

Oligodendrogliomas arise from oligodendroglia cells. They represent 25-33% of glial tumors [160, 161] and have an incidence peak at 40 years, with a smaller earlier peak in childhood between 6-12 years [162]. They show a slight preference for male, with a male-female ratio of 3:2. In 90% of cases they are supratentorial. Surgery is the treatment of choice; chemotherapy is recommended, whereas XRT is suggested only for anaplastic variants [163]. The median survival for surgically treated lesions is 35 months [164], although the ten-year survival is about 10-30% [165]. According to Nurnberg [82] and Hiraga [139], oligodendrogliomas seem to have compatible or longer telomeres than normal brain tissues and detectable telomerase activity. The lack of reduced telomeres in telomerase positive tumor samples can be considered a marker of the less aggressive behavior of such tumors.

Different results have been reported for oligoastrocytomas, that show shorter telomeres than normal brain tissue associated to high telomerase activity [86]. This pattern is more frequent in the anaplastic variant, confirming the role of telomerase activity associated to reduced telomeres as markers of aggressive behavior in these neoplasms as in astrocytic gliomas [139].

5.3. Meningiomas

Meningiomas are extra-axial, usually benign tumors with a slow growing rate that arise from arachnoid cells. They account for 34% of all primary brain and CNS tumors [146]. The incidence peak is at 45 years with a male-female ratio of 1:2.2. About 1.5% occur in childhood and adolescence between 10-20-year age [166]. Surgery is the treatment of choice for symptomatic meningiomas and the five-year survival is 91.3% [167].

Benign meningiomas (WHO grade I) show a variable telomere length [82, 139, 168], sometimes slightly shorter than normal meningeal tissue [145], whereas atypical and anaplastic meningiomas show telomerase activity associated with a significant telomere length shortening [169]. This can be explained considering that the slow growth of benign meningiomas and the absence of telomerase activity cause an equally slow shortening of telomeres, whereas the higher replicative capacity of atypical and especially of malignant meningiomas determines a more evident reduction of telomere length. In these settings, the activation of telomerase activity can give to the cell the capacity to escape from senescence and, consequently, an high proliferative strength. Therefore detectable telomerase activity and shortened telomere length suggest that the tumor contains a cell population with the capacity for unlimited proliferation.

These results indicate that telomere shortening together with telomerase activity may be a critical step in pathogenesis of atypical and malignant meningiomas and may correlate with their malignant behavior. Indeed, as previously discussed for astrocytic tumors, the association of telomerase activity with reduced telomeres length may be considered a marker of an aggressive behavior also in meningiomas, being more frequent in atypical and malignant meningiomas.

5.4. Schwannomas

Schwannomas are benign, usually encapsulated, peripheral nerve sheath tumor composed of neoplastic Schwann cells that can have an intracranial or spinal localization. They account for about 8-10% of intracranial and 25-30% of spinal tumors. The peak of incidence is between the 4th and 6th decades [146]. The vast majority of intracranial Schwannomas develops from vestibular nerve. Tumors of trigeminal or facial nerve are far less common. Management includes many options: surgery, wait and see strategy for little or no growth tumors, radiation therapy alone or in conjunction with surgery, chemotherapy, radiosurgery [170].

Schwannomas usually show reduced telomeres, benign pathological features, low proliferative indices and a lack of telomerase activity [169, 171]. These peculiarities allows such tumors to have a benign clinical course. Conversely, rarely they can assume an aggressive behaviour that is characterized by malignant pathological features and high replicative potential that are usually associated to elongated telomeres. This can be explained considering that long telomeres allow tumor cells to maintain an high proliferative capacity without the need of telomerase activity. When the length of the telomere is long enough for proliferation, the telomerase activity subsides.

For these reasons, telomeres elongation, even in absence of telomerase activity, can be considered a marker of aggressive clinicopathological behaviour in schwannomas [169, 171].

5.5. Primitive Neuroectodermal Tumors (PNETS)

Primary neuroectodermal tumors (PNETs), also called embryonal tumors, represent 1 % of all primary central nervous system (CNS) tumors. They are frequent especially in children, representing the most frequent brain and CNS tumor in the range between 0 and 4 years old [146].

They are highly malignant lesions and can disseminate via CSF spontaneously or iatrogenically [172]. Extranural metastases can also occur. Radical surgical excision is the treatment of choice. XRT is indicated following surgical removal, but it should be avoided at all if possible before 3 years of age to avoid intellectual impairment and growth retardation. Overall survival rate for PNETs is substantially poor with an expected 3 year progression free survival of approximately 50% for localized supratentorial PNETs [173].

PNETs usually show telomerase activity even if there is not a clear association with telomere length. Despite Hiraga [139] and Rahman [174] reported reduced telomeres in the most part of tumor samples, data from Didiano [175] document a wide variability of telomeres length. Interestingly, Rahman reported shorter telomere length in PNETs cells than GBMs, suggesting an higher replicative activity in the former as compared to the latter. These results underline and explain the aggressive behaviour of neuroectodermal tumors. The same author, reported a minority of cases with elongated telomeres and undetectable telomerase activity, suggesting ALT as alternative mechanism of telomere maintenance in these tumors [174].

In summary, telomerase activity can be considered a marker of PNETs and can explain the malignancy of these tumors, although there is not an evident correlation with telomere length. Moreover, in a little percentage of cases without telomerase activity, the aggressive behaviour of PNETs is due to ALT and is associated to elongated telomeres, that allow tumour cells to actively replicate [175].

5.6. Pituitary adenomas

Pituitary adenomas are usually benign tumors that arise from anterior pituitary cells (adenohypophysis). They represent about 13% of primary brain and CNS tumors with an incidence peak on 3th and 4th decades of life [146]. Treatment of pituitary adenomas include medical treatment (dopamine agonists, somatostatin, etc), surgery (transcranial or transsphenoidal approach) or XRT (in case of recurrence that cannot be surgically removed or treated medically).

Their usually benign behaviour can be explained with the lack of telomerase activity, although telomere length is slightly reduced as compared to normal brain tissue [139]. Sometimes they can recur or, rarely, they can progress to pituitary adenocarcinomas. As demonstrated by Harada and colleagues, during progression to carcinoma, the replicative activity of tumor cells causes a progressive shortening of telomeres and the reactivation of telomerase [176]. Therefore, also in pituitary adenomas, telomerase activity and reduced telomere length can be considered marker of aggressive behaviour and can be used to predict the progression to carcinomas.

5.7. Primary Central Nervous System Lymphomas (PCNSLs)

Primary central nervous system lymphomas (PCNSL) also known as primary brain lymphomas are a primary intracranial tumors appearing mostly in patients with severe immunosuppression (typically patients with AIDS). They represent the 0.5-1.2% of intracranial tumours and less than 1 % of extranodal non-Hodgkin lymphomas (NHL), although in the last years there was a progressive increase of incidence also in immunocompetent patients. They affect

all age groups, but are most commonly diagnosed in people who are over 50 years of age. Histologically they are a form of extranodal, high-grade non-Hodgkin lymphoma. Most PCNSLs (about 90%) are diffuse large B-cell lymphomas (DLBCLs); the remaining 10% are poorly characterized low-grade lymphomas, Burkitt lymphomas, and T-cell lymphomas [177]. PCNSLs originate inside the CNS and typically remain confined, rarely spreading outside the nervous system. Although the origin cells are lymphocytes, PCNSLs can be assimilated to brain tumors for their intracranial localization and relationship with brain parenchyma and brain blood barrier that gives PCNSLs the same therapeutic challenges (i.e. in terms of drug delivery through the blood brain barrier) of the other brain tumors.

Data from the literature demonstrate that in PCNSLs have variable telomere length, usually reduced as compared with normal brain tissue, and show high telomerase activity [139, 178].

Harada et al demonstrated a statistically significant correlation between telomerase activity and the survival period of patients with PCNSL. In particular, patients with high telomerase activity had poor prognosis and short survival, regardless of telomere length [178].

These findings suggest that telomerase activity represents a common features in PCNSLs and can be considered by itself a marker for predicting a poor prognosis, regardless of telomeres length, the age at onset and the KPS score before the initial treatment. Nevertheless, telomeres are usually shortened in these tumors and this can explain the aggressive behavior of such tumors, even if the association with patients' survival has not been demonstrated.

5.8. Other intracranial tumors

Only few studies have been performed focusing on the analysis of telomere length and telomerase activity among less common non-neuroepithelial tumors, such as hemangioblastomas, hemangiopericytomas and germ cell tumors.

Hemangioblastomas are benign tumors that usually show normal telomere length and undetectable telomerase activity. Nevertheless, the rare cases of hemangioblastomas with aggressive behavior show shorter telomeres than normal brain tumors and are telomerase-positive [139].

Conversely, malignant non-neuroepithelial tumors such as hemangiopericytomas and germ cell tumors, usually show high telomerase activity associated to an important shortening of telomere length [139].

This suggest that, also for these rare non-neuroepithelial brain tumors, telomerase activity associated to reduced telomere length can be considered a marker of malignancy.

6. Conclusions

There is a desperate need for developing innovative diagnostic tools and therapies for brain tumors.

According to their role in the regulation of cell replication and senescence, telomeres and telomeric proteins represent a new interesting research field to better understand the alterations responsible of carcinogenesis and malignant progression of human brain tumors. In these settings, elucidation of telomeres biology represents the new frontier of cancer research. Although a lot of studies have already been focused on this promising field, further studies should be performed to better understand the pathways involved in the telomeres length maintenance and, consequently, in the process of carcinogenesis and malignant progression of human brain tumors, in order to discover new diagnostic/prognostic tools or new therapeutic strategies for improving prognosis of patients affected by these terrible neoplasms.

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