Chapter 17

Chromosomal Analysis: Clinical Applicability to Brain Cancers

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Additional information is available at the end of the chapter

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1. Introduction

Cytogenetics is the branch of genetics that studies the cell activity focusing mainly on the chromosome structure, organization and function, isolated or as the whole karyotype, in order to understand aspects of cell biology, evolution or implicated diseases. The behavior of DNA and genes is greatly constrained by the fact that they are incorporated into chromosomes. The DNA is associated with proteins that control and catalyze the processes of transcription and replication. Gene expression is controlled by modifications in histones and by chromatin remodeling complexes. It can also be influenced by the position of the gene in the chromosome. Hence, errors in chromosome behavior are an important cause of ill-health. The presence of chromosomal abnormalities is usual in cancer, and specific chromosome abnormality may often be one of the first events in the development of cancer [1]. The importance of cytogenetic analysis in oncology is demonstrated by the number of researches made on this area since the discovery of the Philadelphia chromosome, a 9/22 translocation, which is seen in chronic myelogenous leukemia (CML) patients [2]. The focus of these studies is the relation between specific chromosome alterations to prognosis, drug resistance and diagnosis for some tumors entities. Moreover, DNA repair problems and others genomic stability pathways defects may lead to genome-wide genetic instability, which can drive further cancer progression [3]. Although chromosome rearrangements are mainly used as markers in hematologic cancers, these alterations have been increasingly studied in solid tumors (90% of all human malignancies), showing that chromosomal numerical/structural aberrations are common in this kind of neoplasia.

Brain cancers are very diverse solid tumors that demonstrate a wide range of complex karyotypes. The chromosomal features of each tumor can provide information that helps in

clinical decisions, stratifying it in low and high risk in complementation to the grading aspects usually considered to the central nervous system (CNS) cancers [4]. On this chapter we will consider the implications of the presence of some chromosome mutations for specifics brain tumors. How can these specific alterations help in risk stratification? How these aberrations influence on the choice of treatment? What these rearranged chromosomes indicate about recurrence, metastasis, overall survival or resistance? Obviously, chromosomal mutations have many implications to the cell behavior, affecting the gene dosage by a deletion or amplification, or driving the formation of chimerical transcripts because of chromosomal translocations, etc. Each chromosomal rearrangement has an effect in gene expression or global metabolic equilibrium of the cells. The variety of chromosomal rearrangements is great, involving numerical and structural, and also including very specific types found mainly in cancer, such as double minutes (DMs) and homogeneously staining regions (HSRs), which correspond to gene amplifications (Figure 1).

The importance of chromosomal studies in brain tumors is highlighted by the fact that the most recent World Health Organization's (WHO) book of the CNS neoplasm classification [4] has improved the knowledge about the tumors entities with molecular and cytogenetic markers that, together with the histopathology features, helps in identifying, stratifying or understanding the behavior of tumors.

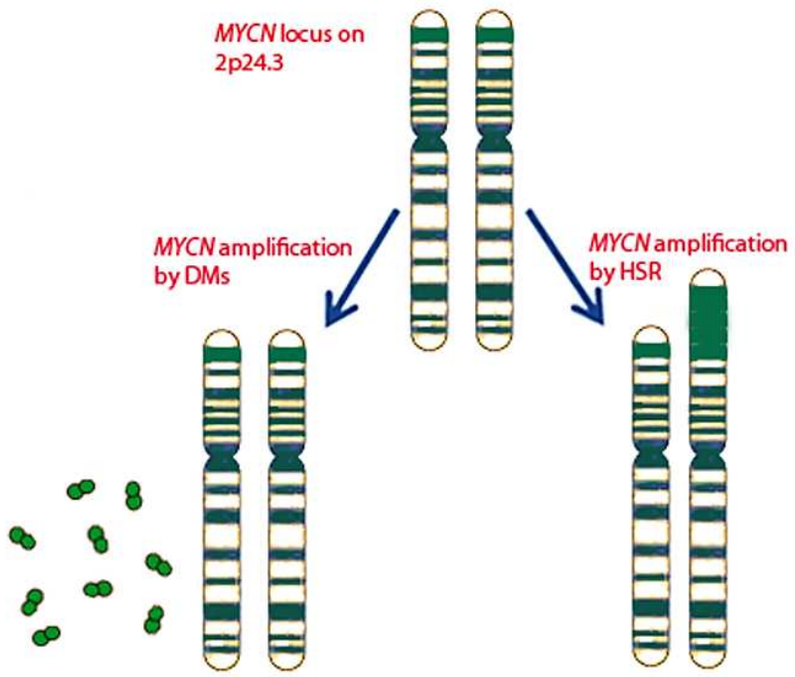

Figure 1. Amplification represents one of the major molecular pathways through which the oncogenic potential of proto-oncogenes is activated during tumorigenesis. In the example, *MYCN* (a MYC family gene) on 2p24.3 is showed amplified by two different mechanisms: extra-chromosomal amplifications (double minutes – DM), and intrachromosomal amplifications (Homogeneously Staining Region (HSR).

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2. Cytogenetics and cancer

The idea that chromosomal rearrangements might be causally involved in early stages of carcinogenesis is not new. The first reports hypothesizing that karyotypic aberrations, typical of tumor cells, may possibly be involved in the transformation of normal cells into malignant ones was published more than a century ago by Theodor Boveri [5] in 1914. Although limited by the poor techniques and the restricted knowledge of cell biology, those early findings allowed him to formulate what is now known as the somatic mutation theory of cancer, which still holds the central stage of cancer research [6]. Because cancer cells usually exhibit abnormal karyotypes, a number of questions have emerged: are these abnormal chromosomes a cause or a consequence of tumorigenesis? Can a single gene mutation drive the neoplastic transformation? One assumption is correct: Some cytogenetic alterations have demonstrated that they are directly linked to tumor formation, progression or metastases, as they are found since the very beginning of tumorigenesis. The observation that some genes affected by chromosomal rearrangements were involved in critical stages in cell growth, development, or survival has focused the interest on how these rearrangements alter the function of target genes. These studies have led to a better understanding of origin of chromosomal alterations and their role in cancer development.

It is widely accepted that the process of tumorigenesis is initiated by an acquired mutation that confers a selective advantage on a dividing cell. This mutated cell will be exposed to new mutations and each new mutation will be passible of a new round of Darwinian selection [7]. The cell genome is far from stable, with intrinsic errors in replication, checkpoint, repair, apoptosis, chromosome segregation, recombination, etc. Some of these mutations can guide to chromosomal instability (CIN) and consequently to higher tendency to cancer formation [8], a situation well illustrated in patients with repair process mutation syndromes, such as *Xeroderma Pigmentosum* and Fanconi anemia, which are associated with an increased risk of cancer.

Albertson and coworkers [9] affirmed that the importance of chromosomal aberrations to tumor development varies substantially between tumors. While there are some tumors with marked chromosome rearrangements, others may evolve by mechanisms that result in little chromosomal change. The difference resides on tumor initiation or the manner in which genome instability is formed. In the same way, the ratio of chromosome rearrangement is accompanied by the malignant stage evolution of a neoplasm, and those pre-malignant tumors show few chromosomal aberrations, which are substantially increased on the malignant ones, supporting a role in chromosomal aberration acquisition in tumor progression. An important advance in the study of chromosomal rearrangements, especially aneuploidy, was the discovery that many cancer cell lines exhibit CIN, a phenotype in which cell division is accompanied by an abnormally high rate of chromosome loss and gain. Thus, CIN can be considered as one form of genomic instability, along with elevated rates of mutation, errors in DNA repair and somatic hyperrecombination [10, 11].

Many studies have also focused on the elucidation of the differential response to treatment of cancers from the same histological classification. Because resistance for multiple drugs cannot

be explained solely in the light of gene mutation, Duesberg and coworkers [12] developed a theory in which they affirm that the dynamic evolution of karyotypes in cancers cells can be responsible for resistance acquisition to most drugs utilized on treatments of cancers. This karyotype evolution is derived by rounds of chromosome mutations facilitated by CIN, followed by Darwinian selection which increases oncogenic functions in cells. It can explain the rapid evolution of a tumor to gain resistance to drugs administrated on the chemotherapy transforming itself autocatalytically. The natural selection drives the constitution of some chromosome mutation to give a selective advantage to cell not only in growth but also in resistance to drugs and other features of the cancer environment. It is not surprising that the presence of chromosomal abnormalities in malignancies has been pivotal in the discovery of targeted therapy against cancer cells, or in discriminating patients sensitive or insensitive to traditional or new therapies [8]. Today we can say that chromosome abnormalities can be used as markers in many different types of malignancy in the cases when it is observed that specific rearrangements are found in tumors with a certain behavior or grade. In many different kinds of tumors, the presence of a specific chromosomal abnormality has improved the quality of the diagnosis, allowing a clearer definition of the prognosis and permitting the definition of new targets on cancer therapy.

The origin and progression of cancer always were unsolved questions to majority of tumor types. Li and coworkers [13] accompanied the chromosomal alterations in human cell lineages for many generations after transformation by SV40 aneuploidogenics genes. They proposed a theory in which cancer-causing karyotypes represent chromosomal equilibria between destabilizing aneuploidy and stabilizing selection for oncogenic function. Furthermore, they concluded that karyotypes are more likely to initiate and maintain cancers than specific gene mutations (Figure 2). One of the great questions concerns the quasi-stable karyotype observed in different passages of a long time cultured cell lineage or between different samples of the same tumor type versus de CIN observed on cancer cells. The answer comes from the Darwinian selection to give oncogenic function to cell, much like new species. Thus, this tumor karyotype increases and maintains the CIN and can form nonneoplastic and nonviable karyotypic compositions which will be eliminated (Figure 2: B, C and D); however, it can evolve to a karyotype that provides new capability to cells such as drug-resistance or metastasis (Figure 2: C, D and E). So, they defined two steps to cancer initiation: 1) the chromosomal instability initiation by carcinogens that generate random aneuploidy and 2) the karyotype Darwinian selection to give oncogenic function, that emerge from unstable randomly aneuploid karyotypes. In our opinion, Li's theory of cancer-causing karyotypes is very concise and is supported by different studies. Routine cancer cell culture experience demonstrates easily that tumor samples show different karyotypic compositions, with clonal structural or numerical chromosomal abnormalities, which are examples of CIN with the so called selection for oncogenic function. In complementation, oncogenic chromosomal compositions can be maintained quasi-stable in distant passages of a cancer cell culture.

The recognition of the importance of cytogenetic science to cancer surveillance has accompanied the technological development of microscopy, computer image acquisition's software and fluorochrome applications. Although chromosome-banding is still the gold standard for

all routine techniques of clinical and tumor cytogenetics, the technical restrictions of this methodology are well known. Only changes that affect the normal pattern such as size variations or position in a chromosomal band or the chromosome itself can be detected, and the origin of additional material or gain/loss of small amounts in a structurally altered chromosome often remains questionable. To overcome such limitations, fluorescence *in situ* hybridization (FISH) approaches were introduced into cytogenetics. FISH is a technique based on the probe-sample hybrid formation labeled with a detectable fluorescent dye. It is a reliable technique that has made a revolution to chromosome mutation detection. Many other techniques have derived from FISH: Interphase FISH, chromosome region specific FISH (telomere, centromere, etc.), multicolor FISH, SKY, Multiplex-FISH, CGH, array-CGH, microarray-CGH, FISH banding, etc. In general, these techniques are very informative and can be utilized in complementation to those classical clinical histopathology diagnostic procedures.

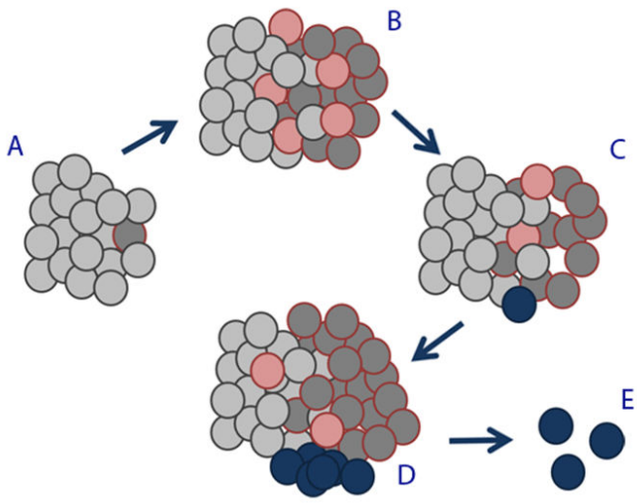

Figure 2. Li's theory of cancer-causing karyotypes. (A) Cells with CIN (gray cells) generate karyotypes with oncogenic functions (dark gray cell). (B) Cells with oncogenic functions will develop and grow forming nonneoplastic and nonviable cells (pink cells), which will be continually generated and eliminated (C and D). However, some karyotypes evolve to more aggressive behavior, like metastatic or drug-resistance cells (blue cells) which can migrate to others sites (C, D and E).

Some of these techniques, such as SKY, M-FISH or CGH-based methodologies (Figure 3), are able to show all chromosomal alterations of a sample in a single experiment, each respecting its limitation, of course. SKY and M-FISH (Figure 3A) can differently dye every chromosome pair in a metaphase spread of a tumor sample, using five different fluorochromes in 24 distinct combinations (22 autosomes, X and Y). These techniques allow the definition of the origin of each chromosome segment involved in rearrangement, but cannot indicate microdeletions or the gain/loss of specific loci. The CGH-based methodologies (Figure 3B) use only two different fluorochromes, for cancer and negative control, showing all losses, gains and amplifications in a tumor sample. These technological advances had led to an exponential increase on the number of patients cytogenetically analyzed. After decades of cancer genome and karyotype

analysis, is estimated that 14% (about 3000) of the human genes are involved on cancer formation and progression [8], and the quantity of chromosome alterations involved is equally large. Therefore, some web tools were created to permit a faster search of these genic and chromosomal mutations on cancer for specific entities, as for example the "Mitelman Catalog of chromosomal alterations" [14].

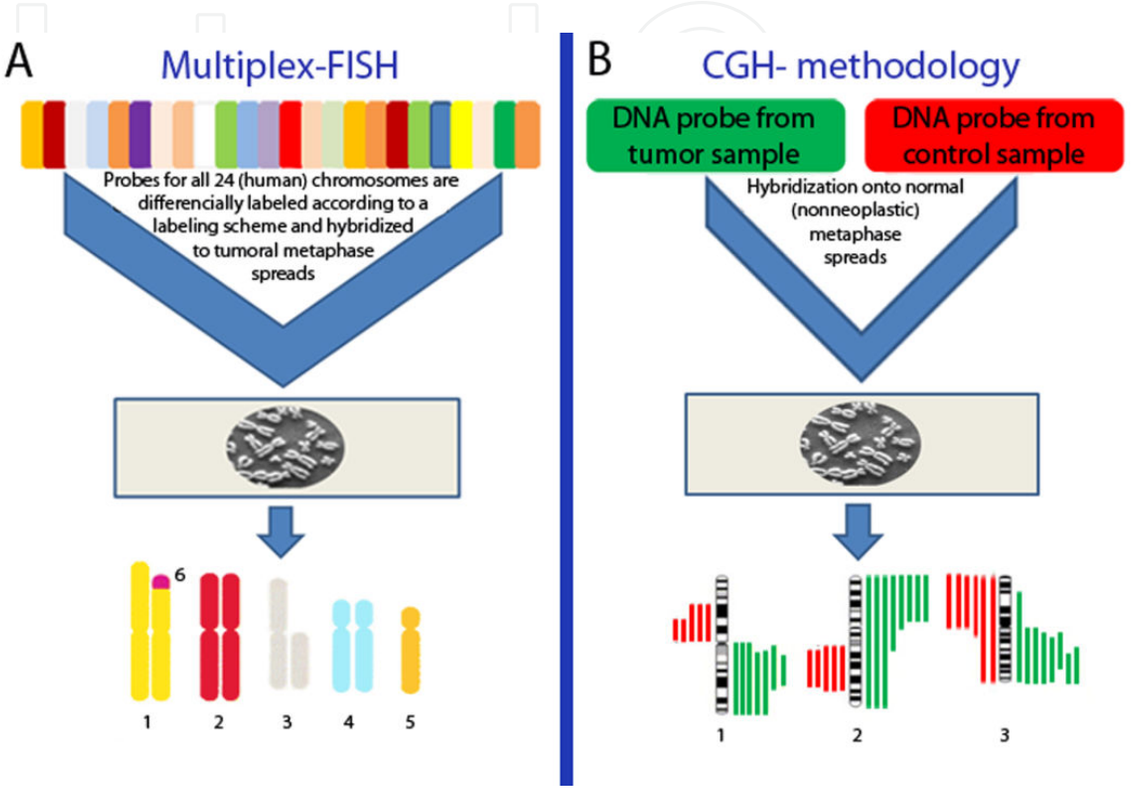

Figure 3. Whole-genome analysis of a tumor cell can be obtained by (A) M-FISH, SKY or (B) CGH methodologies. (A) SKY or M-FISH utilizes a pool with 24 differently labeled chromosomes with a combination of five distinct fluorochromes to show all chromosomal rearrangements in a metaphase, including structural and numerical rearrangements and markers chromosome. (B) CGH utilizes two different DNA probes, from tumor and from a control nonneoplastic sample, to hybridize onto a spread metaphase, or more recently in a slide array, to demonstrate DNA loss, gain or amplification in the tumor sample.

Further down we will list some of the new and well established correlations between brain tumors entities clinical behavior and some specific chromosome abnormalities. Some of the imbalances were correlated with particular pathways or genic imbalances, others have no well-established correlation with a specific gene function disturbance or cell pathways modifications, although are systematically found in some brain tumor types.

3. Brain tumors

Brain tumors are the second most common type of cancer in children and are associated with poor survival both in infants and adults, representing, therefore, a heavy burden for the patients and their relatives [15]. These tumors can be devastating because they are difficult to

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treat, and frequently cause mental impairment or death. The incidence of brain tumors has increased during the past three decades for all age and gender groups as a result of imaging with computed tomography (CT) and magnetic resonance imaging (MRI), lymphomas secondary to HIV/AIDS, and changes in coding and classification [16]. With the exception of meningiomas and pituitary adenomas, women are less likely than men to be diagnosed with a brain tumor, particularly glioblastoma multiforme and anaplastic astrocytoma, as well as medulloblastomas. The lifetime risk of contracting a primary malignant brain tumor is 0.52% for women and 0.67% for men. Similarly, the chance of dying from a malignant brain tumor is 0.40% for women and 0.49% for men. There have been growing amount of studies dedicated to detecting chromosomal imbalances and intercellular genomic variations both in benign and malign brain tumors. Currently, it is suggested that almost all the chromosomes are involved in aberrations associated with brain tumorigenesis [17]. Moreover, some specifics chromosome structures can differentiate the tumor grades, in accordance to WHO classification, simply by determining its proliferative potential, it is the case of Nucleolar Organizer Region (NOR), which can be analyzed by AgNOR to discriminate benign and malignant brain tumors [18]. Nevertheless, there are a number of chromosomal regions that are recurrently rearranged in brain tumors.

In order to illustrate the importance of cytogenetic studies and the relation of some recurrent rearrangements and tumor behavior/classification, we are going to describe the most common chromosomal rearrangements in some brain tumors.

4. Gliomas

The most common malignant primary brain tumors are gliomas, corresponding to more than 70% of the total primary brain tumors. They include a variety of malignant grades and histological tumor types. Gliomas can be classified in Astrocytic tumors, oligodendrogliomas and Ependymal tumors in accordance with the WHO classification of the tumors [4]. The most common gliomas are astrocytic tumors in which the most malignant entity is the glioblastoma (WHO grade IV). Gliomas are characterized as non-curable tumors. Today histopathology is still the gold standard for diagnosis and grading of gliomas tumors. However some markers have emerged and have important applications to their classification and prognosis.

4.1. Astrocytic tumors

The Astrocytic tumors are very diverse and represent the largest and most common group of brain tumors. The Astrocytomas, Anaplastic Astrocytomas and the secondary Glioblastomas (GBMs) are examples of tumors that show linear progression from benign to malignant neoplasms [4]. This progression is driven by some specific genetic events including chromosomal mutations. The most common cytogenetic observation is an increased complexity of the karyotype, both structural and numerically, concurrent with the progression in malignancy. Amplifications of *EGFR* locus on 7p12 and *PTEN* mutations on 10q23.3 are the best known genetic markers that distinguish the *de novo* GBM from GBM that has a progression from a low

grade astrocytic tumor, which is frequently associated with *TP53* and *IDH1* gene mutations [19-21]. Trisomy of 7 and monosomy of 10 as well as frequent gains of 12p, 19q, and 20q differ primary from secondary glioblastomas [22]. Despite of these cytogenetic differences, primary or secondary glioblastomas can be assigned to a common set of functional pathways [23].

EGFR-mediated signaling is up regulated in about 30% of malignant gliomas and 60% of GBM [24-26]. In GBM the overexpression generally is driven by *EGFR* gene amplification [27, 28]. Several contradictory studies have been made in prognostic value determination of *EGFR* amplification [29]. Erlotinib and gefitinib are two drugs that target the *EGFR* amplification/overexpression positive patients. These drugs presented unclear results up to date, with different researches demonstrating contradictory results. However, a recent study showed that co-expression of *PTEN* and *EGFRvIII* (a mutant form of *EGFR* molecules that constitutively activates the EGFR-phosphoinositide 3-kinase pathway) was associated with an increased sensitivity to erlotinib, whereas tumors without *PTEN* expression did not respond to erlotinib [30]. In another study, glioblastoma patients treated with these drugs did not show major response or survival improvement [31, 32]. On the other hand, a mixed result was obtained by the combined use of erlotinib with temozolomide and radiotherapy [33, 34]: a group with no overall benefit that did not help to identify a subgroup of tumors that might respond to therapy [33], and other group suggested that this regime might be useful for patients with tumors with *MGMT* promoter methylation and intact *PTEN* [34]. There is no consensus about use of these drugs in gliomas and glioblastomas patients to date, but new drugs and new molecularly targeted drugs reached clinical trials [35].

A more informative scenery is obtained by the simultaneous analyses of *EGFR* gene amplification and *EGFRvIII* in gliomas. An estimative of 50-60% of the amplified *EGFR* patients presents the *EGFRvIII* mutation. This condition is considered both diagnostic and prognostically informative, indicating a high grade malignancy. It is suggested that anaplastic or low-grade gliomas with this combination are more malignant than indicated by their histopathology and an unfavorable impact on the prognosis has been described for these patients [36]. To high grade gliomas, like primary glioblastomas, the *EGFR* amplification/*EGFRvIII* poor prognosis is less obvious [37] but some studies have reported a poor prognosis association [38-41].

In glioblastomas, *EGFR* amplification mostly occurs as double-minutes (DM), which are small fragments of extra-chromosomal DNA. FISH assessment of this amplification is an accessible technique to be made in conventional pathology laboratories, which can be made in interphasic nuclei of a paraffin-embeded section [42, 43]. Quantitative PCR or reverse transcriptase PCR can be used to detect *EGFR* amplification as well [44]. Immunohistochemistry is used, but its value is less clear [45].

Some alterations in astrocytomas can indicate an increased risk of dying, independently of its histological grade, such as the presence of +7q and -10q chromosomal alterations detected by the CGH analysis of astrocytomas [46]. Misra and coworkers [47] identified three groups in GBM patients: those with both 7 gain and 10 loss, some with 10 loss without gain of 7 and the group without these two alterations. In clinical evaluation, the patients with 7 gain and 10 loss showed typical characteristic of GBM short-term survivors. In contrast, patients who had none

of these alterations showed characteristics of typical and long-term survivors. In this research it was showed that *EGFR* is amplified on 7 gain 10 loss group of primary GBM. In a review using interphase-based FISH to chromosomes 7 and 10, it was found that 75% of the astrocytomas grade II and 100% of the grade III and IV exhibited cells with polysomy of chromosome 7 and that 75% of the grades II and III or 100% of the GBM samples showed cells with loss of chromosome 10 [20].

A gene expression profile (GEP) in a series of gliomas was associated with the cytogenetic of the glioblastomas and with the histopathology of gliomas [48]. When low-grades versus high-grades gliomas were compared, divergent profiles both cytogenetic and GEP were exhibited. High grades gliomas demonstrated higher intratumoral cytogenetic heterogeneity (demonstrated by a higher number of cell clones). The authors correlated this with the genomic instability or with the ancestral tumor cell clone chromosomal alterations in which karyotype composition led to an increased CIN. According to this study, three distinct glioblastomas GEP groups were formed: GEP1 with *EGFR* amplification, GEP2 with isolated trisomy 7 and GEP3 demonstrating more complex karyotype. All these three groups were formed after analysis of ancestral tumor cell clone and further cytogenetic evolution of the tumor cells of GEP1, GEP2 and GEP3 glioblastomas were related with 7 gain, 9p and 10q deletions, suggesting a simultaneous occurrence of *EGFR* activation (normal or mutant variants) and loss of both *Ink4A*/*Arf* and *PTEN* tumor suppressor genes.

4.2. Oligodendroglial tumors

Oligodendroglial tumors are diffusely infiltrating, well differentiated gliomas, typically located in cerebral hemispheres in adults, composed of neoplastic cells morphologically resembling oligodendroglia [4]. In oligodendrogliomas (WHO grade II) 80-90% are correlated to simultaneous deletion of 1p and 19q, whereas more malignant tumors demonstrate lower frequencies of this same alteration. The anaplastic oligodendrogliomas (WHO grade III) present 1p/19q co-deletions in approximately 50-60%, oligoastrocytomas in 30-50%, anaplastic oligoastrocytomas in 20-30%, and diffuse astrocytic gliomas in less than 10%, including glioblastomas. Currently, loss of 1p and 19q is the genetic hallmark of oligodendroglial tumors [4, 49]. Theses deletions were firstly associated with PVC (Procarbazine, CCNU and vincristine) sensitivity, demonstrating a favorable outcome in contrast with patients who don't show these chromosomal deletions, simultaneously [50]. Nowadays, this substantially improved survival times was correlated with others drugs (like temozolomide) or procedures (like radiotherapy) sensitivity, suggesting that 1p/19q co-deletion is an indicator of tumor vulnerability to a broad range of therapeutic options than as a specific predictor of chemosensitivity [35].

Interestingly, although the 1p and 19q regions have been extensively mapped, no tumorigenic gene was implicated. Another observation is that 1p/19q co-deletion tumors generally present a classical histology [51-53] and is correlated with *IDH1* and *IDH2* mutations [54]. On the other hand, *TP53* mutation, 10q deletions and *EGFR* amplifications were inversely correlated with 1p/19q co-deletion tumors [53]. Another association is obtained from tumor location: when anaplastic oligodendrogliomas and low grade oligodendrogliomas occur in the frontal,

parietal, and occipital lobes, they are generally related to 1p/19q co-deletions [55-57]. In glioblastomas 1p/19q co-deletion is uncommon, however, when it is detected, the results observed are opposite, predicting shortened survival [52]. Thus, this cytogenetic marker denotes a clinically distinct tumor, with progression, prognosis, and treatment responses that are different of others gliomas. Therefore detection of 1p/19q alterations in oligodendrogliomas has become a useful and common test procedure [35]. FISH is the most reliable procedure to detect this marker in the laboratories, which can be substituted by array CGH in the next future when this technique may become less expensive.

By contrast, when the short arm of chromosome 1 is deleted alone, which is a rare cytogenetic finding in gliomas, it is associated with a poorer prognosis. On the other hand, in glioblastomas, primary or secondary, loss of heterozygosity (LOH) of 1p (other rare observation) is associated with longer survival [50]. In contrast, the oligodendroglial tumors are associated with poorer outcome when 8q gains are observed [20].

4.3. Pediatric gliomas

Pilocytic astrocytomas commonly present a characteristic *BRAF* proto-oncogene activation at 7q34, mainly by gene fusion or duplication, which is infrequent in diffusely infiltrating astrocytic gliomas [58-61]. Therefore, difficult diferential diagnosis between pilocytic astrocytoma and low-grade diffuse astrocytoma could be improved by the detection of *BRAF* activation. *BRAF* is target of a new therapy that inhibits the MAPK pathway, as showed in a case report [62]. The detection of *BRAF* fusion can be made by specific FISH probes or by specialized RT-PCR assays.

5. CNS Embryonal tumors

Embryonal tumors of the CNS form by far the largest group of malignant brain tumors in childhood. They are characterized by a mass of cells that begins its growth in the embryonic tissue in the brain. Despite the progress in the knowledge of these tumors, few studies were translated on clinical improvement. The WHO classification divides embryonal tumors into three entities: Medulloblastomas, CNS Primitive Neuroectodermal tumors (PNETs) and Atypical teratoid/rhabdoid tumor (AT/RT) [4].

5.1. Medulloblastomas

Medulloblastomas (MBs) are the most frequent embryonal tumors and the most frequent CNS tumor in childhood. They affect the cerebellum and are defined as grade IV in the WHO classification. Histopathological classification differentiates five distinct medulloblastoma variants: the classic MB, desmoplastic/nodular MB, MB with extensive nodularity, anaplastic MB and large cell MB [4].

In a recent comprehension made by [63], MB comprises four distinct molecular subgroups: WNT, SHH, group C and group D. This subgroup classification was made in ac-

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cordance with its GEP of important genes in medulloblastoma disease. The authors also created an immunohistochemistry (an easier methodology to install on the conventional histopathology laboratories) four-antibody approach to discriminate the medulloblastoma patients into the four distinct molecular variants. Children patients classified as group C demonstrate a marked reduction in survival regardless of it metastatic stage. After a recent discussion about the classification of MB in the light of its transcriptome, involving researchers of different laboratories and countries, these subgroup were renamed, to WNT, SHH, Group 3 and Group 4 [64].

Analyzing the somatic copy number aberrations (SCNA) of the MB, Northcott and coworkers [65] concluded that SCNA in MB are common and are predominantly subgroup-enriched. Only the WNT subgroup demonstrated no significant deletions and a small subset of focal gains, which were found in a proportional frequency in non-WNT tumors, concluding that there are no frequent, targetable SCNA on this subgroup. SHH tumors, however, exhibit multiple focal SCNAs restricted/enriched on this group and have important clinical implications [65]. Group 3 and Group 4, which were generically named because less is known about its biology [64], presented important SCNAs restricted/enriched on them. *MYC* amplification (Figure 4A) mutually with *OTX2* oncogene demonstrated that are largely restricted to Group 3, and were extremely prognostic. Furthermore, TGF-β signaling is the unique restricted pathway involved in group 3 tumors, which may indicating a new therapy for Group 3 patients that present a dismal prognosis. In group 4 MB patients, the NF-kB pathway could represent a rational therapeutic target, because *NFKBIA* (14q13) and *USP4* (3p21.3), regulators of NF-kB, were consistently deleted on this group [65]

The most frequent chromosomal abnormality in MB is the isochromosome 17q (i17q), found in approximately 30%-50% of patients [66, 67]. The i17q structure consists of two centromeres, two very centromeric "17p" region that are fused together, mainly in the Smith-Magenis region, and two copies of 17 long arm. It was observed in increased levels of recurrent meduloblastomas compared with the initials ones, suggesting a role in progression of medulloblastomas [68]. But, although there are well-known tumor suppressor genes and oncogenes on chromosome 17, the tumor suppressor genes on the 17p or the oncogenes in the 17q directly involved on MB disease were not yet identified, however the tumor growth advantage may occur by haploinsufficiency for genes on 17p and an increased expression of genes on 17q driven by the copy number alterations. For this reason, 17q gain, 17p loss or both represent the same biological effect of an i17q (Figure 4C), which is an alteration commonly seen in MB patients [63, 68, 69]. The presence of this abnormality was the unique chromosomal alteration that occurs at a high frequency in [63] and was significantly prominent in Group D (Group 4) molecular subgroup of MBs (65.7%). The others chromosomal alterations were seen at a low frequency [63]. The monossomy 6 in the same study and others was detected exclusively in WNT tumors [63, 70-73], while the 9q loss was detected only in SHH tumors [72]. These and others chromosomal markers presents on the four molecular subgroups will be shown in Table 1.

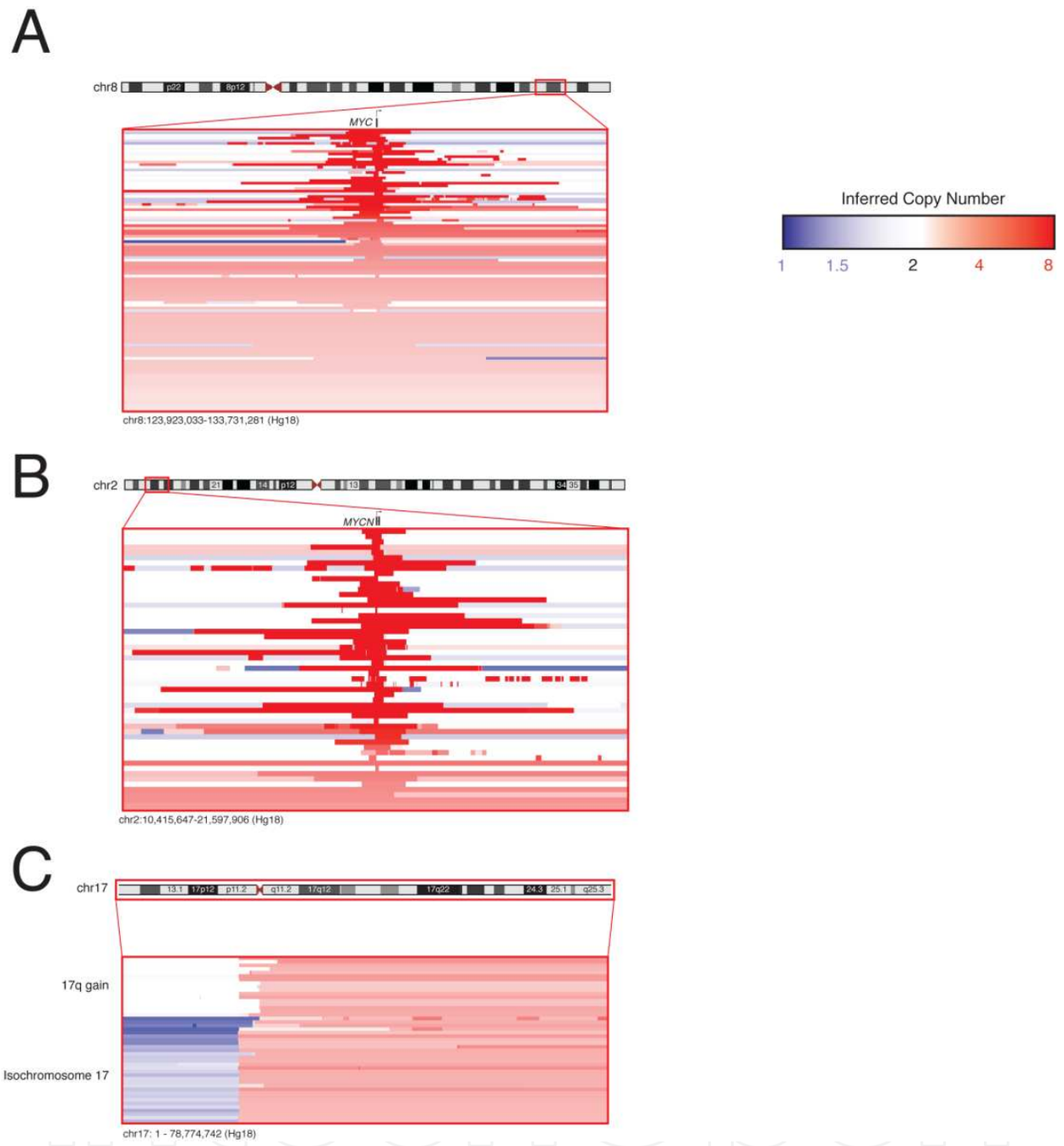

Figure 4. Somatic Copy Number Aberrations (SCNA) in medulloblastomas involving chromosomes (A) 8, (B) 2 and (C) 17. The colors represent the inferred copy number for each chromosome locus, in which red spectrum represent gains/amplification and blue spectrum represent loss/deletions, normal SCNA is represented in white. (A) Chromosome 8 SCNA exhibiting amplification on the *MYC* locus on 8q24.21. (B) Chromosome 2 SCNA showing amplification on *MYCN* locus on 2p24.3. (C) Chromosome 17 SCNA demonstrating 17q gain and both 17p loss and 17q gain (representing i17q). Courtesy of Dubuc AM, Taylor MD, Northcott PA and Shih D. See [65] for details.

The investigation of *MYC* and *MYCN* locus provided consistent prognostic information to medulloblastoma patients [66, 74-79], and can be accessed by FISH or CGH experiments (Figure 4A and 4B), being related mutated in up to 10% of medulloblastomas. *MYC* amplifi-

cation is associated with poor prognosis and with the large cell/anaplastic medulloblastoma variants, but histologically aggressive cases diagnosed, like large cell/anaplastic MB without amplification of *MYC*, were not significantly associated with worse outcome [68]. *MYCN* amplification (Figure 4B) is associated with poor prognosis and correlated with large cell/anaplastic variants, but is clinically more heterogeneous than *MYC*. The MYC family was considered in Northcott molecular stratification of MBs [63, 65], and *MYC* amplification on 8q24 (Figure 4A) was detected exclusively on Group C (Group 3) patients. MYCN was demonstrated amplified (Figure 4B) both in SHH and Group D (Group 4) MB patients [63, 65].

	Losses	Gains	Others
WNT	-	-	Monossomy 6
SHH	9q, 10q, 14	2, 3q, 9p, 20q, 21q	
Group C	5q, 8p, 10q, 11p, 16q	1q, 17q, 18	i17q,
Group D	X, 8p, 8q, 11p	17q, 18	i17q,

Table 1. Significant chromosomal abnormalities observed on the four distinct molecular variants of the Northcott study.

For adult Medulloblastomas, different genetic and cytogenetic changes were observed in relation to pediatric ones, with profiles of chromosomal abnormalities greatly differing from childhood. *CDK6* amplification, 17q gain and 10q loss were strongly associated with shortened survival. The WNT signaling pathway activation does not demonstrate the excellent prognosis seen in pediatrics MB [80].

5.2. Atypical teratoid/rhabdoid tumor

Atypical teratoid/rhabdoid tumors (AT/RT) are very malignant embryonal neoplasms (WHO grade IV) that occur in very young children [4]. Very constant alterations in *SMARCB1* locus on 22q11 were published. These alterations can be detected like deletions, loss of heterozygosity (LOH) or gene mutation in all the exons of this gene. SMARCB1 protein immunohistochemistry search has demonstrated great utility in diagnosis of AT/RT or in determinate patients with poor therapy response and aggressive clinical course, even in the absence of AT/RT cell [81, 82]. When combined FISH, genomic sequencing, MLPA and SNP-based oligonucleotide arrays were used to diagnosis AT/RT in 36 patients, all demonstrated biallelic alteration in *SMARCB1* locus [83]. The molecular diagnostic became yet more important when it is possible to determinate adult carriers, to genetic counseling finalities.

5.3. Ependymoblastomas

Ependymoblastoma and ETANTR (Embryonal Tumor with Abundant Neuropil and True Rosettes) are rare and very aggressive Primitive Neuroectodermal Tumors (PNETs) characterized by the presence of multilayred rosette [4]. They were recently associated with focal amplification of 19q13.42 that contains a cluster of mi-RNA-coding gene. This amplification

was seen in virtually all the embryonal brain tumor with true multilayered rosettes [84-86]. These results indicate that they may represent a single biological entity that can be diagnosed by the detection of 19q13.42 amplification. The term Embryonal Tumor with Multilayered Rosettes (ETMR) was proposed to designate these entities that apparently affect only children and have a very poor prognosis.

6. Ependymal tumors

Ependymomas form a group of heterogeneous tumors anywhere along the craniospinal axis that can occur in adult or childhood. They can originate from the radial glial cells [87, 88] which originate the ependymal cells during normal cellular development. The WHO classification [4] designates ependymal tumors in different histology entities, as hereafter: Subependymomas and myxopapillary ependymomas (WHO grade I); Classic ependymomas (WHO grade II); anaplastic ependymomas (WHO grade III). The classic ependymoma was subdivided in four variant cellular, papillary, clear cell and tanycytic [89]. For these tumors the WHO grading was the most powerful prognostic factor in adult population. In the same way, the tumor location has been demonstrated as having potential prognostic value, with those in the supratentorial regions demonstrating poor prognosis and with higher risk of recurrence.

The chromosomal abnormalities reflect the heterogeneity of topology and age of Ependymomas. A study made by Korshunov and coworkers [90] has presented a comprehensive work that subdivides the ependymomas in three groups:

a. Group 1: five years of Overall survival of 100% - tumors with gain of chromosomes 9, 15q, or 18, or loss of chromosome 6, without 1q gain or *CDKN2A* deletion.

b. Group 2: five years of Overall survival of 78% - tumors balanced for chromosome 1q, 6, 9, 15q, and 18, without a homozygous deletion of *CDKN2A*.

c. Group 3: five years of Overall survival of 32% - tumors with 1q gain or homozygous deletions of *CDKN2A*.

Group 1 demonstrates an excellent response to standard therapy protocols, demonstrating an excellent prognosis. Group 3 demonstrates a propensity to generate metastasis and generally show an aggressive clinical behavior, having its chromosomal composition associated with a poor prognosis. Another possibility for this group is the association of 1q21.1-32.1 gain correlated with an increased propensity to recurrence.

Some chromosome alterations remain unclear between the groups. The 6q23 loss in group 1 can be correlated with a decreased progression-free survival, while 6q25.3 loss in anaplastic ependymomas has been correlated with an improved overall survival. The 9q gain in pediatric group 1 patients was correlated with a frequent recurrence.

Yang and coworkers [91] conclude that diagnosis based only in the light of histologic procedures may be insufficient to assign an appropriate risk stratification strategy. In our opinion the enlargement of cytogenetic analysis could generate a map of chromosomal alterations on

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ependymomas that would help in creating a personalized treatment for these tumors and indicate targets to avoid growth, recurrence or metastasis.

7. Meningiomas

Meningiomas are the second most common tumor of the CNS in adults. They are classified as benign, atypical or anaplastic corresponding to 80%, 15-20% and 1-3%, respectively, and stratified in grades I, II and III, respectively [92]. Even the grade I meningiomas, with a favorable prognosis under the classical treatment with surgical resection, radiation and chemotherapy, presents an aggressive remaining group which needs molecular or cytogenetic markers to distinct its diagnosis and treatment.

The karyotypes of meningiomas show diversity among the WHO grades. The WHO grade I benign meningiomas rarely exhibit chromosomal aberrations beyond 22q losses. More complex karyotype compositions are seen in higher grade meningiomas with more aggressive behavior. The losses are common to 1p, 10q, 14q and less frequent on 6q and 18q in Atypical and Anaplastic meningiomas. Higher grade meningiomas are characterized by gains on 1q, 9q, 12q, 15q, 17q and 20q. Anaplastic meningiomas have demonstrated losses on 9p with amplification on 17q23 in a higher frequency. Alterations on chromosome 1 always represent important alterations on CNS tumors. In meningioma losses on 1p can be related as a strong indicator of recurrence: only 4.3% of the meningiomas with recurrence are seen with an intact 1p. The presence of deletions of 1p can be related to a strong propensity to recur. LOH on 1p, 10q and 9p are also associated with recurrence propensity. At the same way, 9p losses are associated with anaplastic meningiomas (grade III) with *p14ARF* (encoding p14), *CDKN2B*/*p15ARF* (encoding p15), and *CDKN2A*/*p16INKa* (encoding p16) tumor suppressor genes losses. The most important is the *CDKN2A* impairment causing poorer outcome when compared to patients with intact *CDKN2A* genes. Losses in 14q also are found in meningiomas, and are associated with a worse prognosis. 14q deletions serve as a powerful and reliable prognostic factor indicating tumor recurrence [93].

8. Others brain tumors

Less well understood involvement of chromosome abnormalities are reported for some infrequent tumors of the CNS. The low frequency can explain the low number of cytogenetic studies, but the involvement of a multigroup work to understand them could provide a solution to compile these patients. However, some works have made a suggestive involvement of specific chromosomal alterations in the genesis, development, aggressiveness or response to therapies. For these, a brief description will be made here.

An example is the Olfactory Neuroblastoma (also named Esthesioneuroblastoma), that originate from the olfactory epithelium, that form a group of neoplasm less studied at the cytogenetic point of view, but the first comprehensive study has suggested that the prevalence

of 3q deletion demonstrates that it can be adopted as an early genetic event in Esthesioneuroblastoma and the involvement of deletion on chromosomes 5, 6q, 7q, 11p/q, 15q21 as well as gains of 1p32-34, 1q12, and 2p22-24 can be associated with a metastatic phenotype and a worse prognosis [94].

Rickert and coworkers [95] in a study applying CGH in choroid plexus papillomas and choroid plexus carcinomas made the follow correlation: patients with choroid plexus carcinomas were associated to have a significantly longer survival when +9p and -10q alterations were present.

9. Brain metastases

Brain metastases are tumors that originate outside the CNS and secondarily spread to the CNS via the haematogenous route (metastasis) or by direct invasion from adjacent tissues [4]. Metastatic tumors form a heterogeneous group, in which primary site can be from any location in body. But the frequencies of tumors that metastasize to brain are non-random, because there is an organ tropism to each tumor. However, brain metastases can occur in up to 40% of the cancer patients and represent a major cause of mortality and morbidity in cancer patients; some authors indicate that there are under notification of brain metastases [96]. The most commons primary sites that metastasize to brain are the lung, breast and melanoma with frequencies of 40-50%, 15-25% and 5-20%, respectively. However, melanoma will be not considered here since the *BRAF* gene mutation, it the main molecular marker, can't be detected by cytogenetic procedures.

A consensus is that up to date the role of current chemotherapy with cytotoxic drugs is limited to palliation, and the efficacy depends on the chemosensitivity of the primary tumor [97]. A new possibility is to create a therapy that prevents brain metastasis; it will be possible when targeted therapies to known molecular pathways to brain colonization become clear. This possibility could become a common strategy to those tumors that frequently form brain metastases.

On established brain metastases the therapy mainly consists on the use of whole brain radiation therapy (WBRT). Less frequently targeted agents, either alone or in combination with WBRT, have been investigated in newly diagnosed brain metastases [98].

A correlation can be made to Non-small Cell Lung Cancer (NSCLC). NSCLC patients show activating epidermal growth factor receptor (*EGFR*) mutations in 10-25% of the cases, with the highest prevalence in never-smoking women from East Asia, in up to 55%. Erlotinib and gefitinib, *EGFR* tyrosine kinase inhibitors, have been demonstrated to be useful in patients with brain metastases from NSCLC [99-106]. Nevertheless, Brain metastasis from NSCLC patients with mutant *EGFR* confronted with those wild type *EGFR* have demonstrated an improved overall survival, when receiving *EGFR* inhibitors [107]. Another molecular marker in NSCLC patient, a FISH detectable rearrangement in 2p23 in 4% of patients, the ALK rearrangement can be treated with crizotinib (a specific ALK inhibitor) demonstrating objective response or stabilization of the malignancy [108]. A speculation about a brain barrier

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to crizotinib agent could permit a poorer penetration into the brain leading to a lower efficacy, but no data about any treatment with crizotinib to brain metastasis exist to date [109].

The most informative molecular marker on brain metastases of breast cancer came from *HER2*. Breast tumors positive for *HER2*, triple negative lacking expression of HER2, estrogen and progesterone receptors, or the basal-like subtype form the high risk group that can metastasize to brain. The *HER2* breast cancer patients represent 25% of overall population, and have the highest risk of brain metastases development, especially if estrogen/progesterone negative [110]. A recent work analyzed alterations on chromosome 17 in metastatic brain tumors from breast cancers using a dual color experiment with CEP17 and *TP53* locus specific probe. The result was a high incidence of chromosome 17p deletion in these neoplasms, suggesting a role of 17p loss in the metastatic capability acquisition for breast tumor cells [111].

When breast cancer patients are treated with trastuzumab, a monoclonal antibody that target *HER2*, 25-40% tend to present brain metastasis [112-116], which can increase when compared with trastuzumab-no treated patients [117]. An explanation is that trastuzumab efficiently controls the systemic disease spread [118], associated with a *HER2* propensity to brain colonization [119, 120] and with the trastuzumab decreases penetration through the blood-brain barrier [118]. Recent works have demonstrated that a higher penetrance of trastuzumab into the brain, which could be provided by lesion in blood-brain barrier or increased vascular permeability driven by tumor activity or by radiation therapy, have revealed an improved prognosis to *HER2*-positive patients with brain metastases [116, 121-124].

The *HER2* gene, a member of *EGFR* gene family, is located on 17q21.1 and the amplification can be detected by a FISH experiment. HER2 protein overexpression can be detected by an immunohistochemistry method. Both, FISH or immunohistochemistry, can be made in paraffin-embedded tissues. The higher cost and longer time required to cell scoring in FISH experiments make the immunohistochemistry the most utilized procedure in laboratories, but FISH was demonstrated as more efficient and accurate scoring systems to determine *HER2* amplification than immunohistochemistry [125, 126]. More recently, a study aimed to determine a relationship between HER2 protein expression level or *HER2* gene amplification ratio (by FISH with a *HER2* gene probe and CEP17 probe in a dual color experiment) correlated with the time to brain metastases formation in *HER2*+ advanced breast cancer patients. It showed that HER2 protein expression level detection demonstrated a more sensitive method to determinate the time to brain metastases, shown a shorter time to brain metastases in higher level of HER2 protein expression [127].

10. Conclusions

After analyzing all these cumulative information, one can conclude that chromosomal analysis of brain tumors can strongly improve the clinical diagnostic and prognostic in clinical practice and the knowledge about the biology of brain tumors. This information has helped in the choice of the best therapy in widely studied tumor types, and could help even more. Also, the great number of chromosome abnormalities associated to specific tumor entities improves the search

for target genes or cell pathways that direct or indirect act in tumorigenesis or tumor progression. On the other hand, if Li's theory [13] is true and the tumors are generated by aneuploidogenics carcinogens or mutations, targeted preventions to avoid aneuploidy/aneuploidy cells or a methodology that enhances genomic stability/cell defense mechanisms against cells with CIN could provide an effective approach.

Similarly, it is clear that some chromosomal alterations are more important to a wide range of brain tumor, participating in the genesis, progression, metastases and others hallmarks of cancer than to a specific entity. Alterations on chromosome 1, 7, 8, 10, 17 and 22 appear to be important to a variety of tumors of the brain. 1q gain is an example of alteration that leads to a worse prognosis, correlated with tumor recurrence or progression. At the same way, 17q gains and 17p losses almost always represent a poor prognosis. The presence of important tumor suppressor genes or oncogenes in these loci can explain its higher participation on the evolution of tumors cells to achieve the malignancy. Gain of chromosome 7q could be related with *EGFR* amplification, which is implicated with a large number of brain tumors entities. Likewise 17q gain could be related to *HER2* amplification, or 17p losses could be associated with the selective advantage of *TP53* pathways inactivation.

Obviously, as molecular markers, locus amplifications/deletions, structural abnormal chromosomes or aneuploidies are important genetic mutations that confer to tumors different clinical and biological behavior. These markers can be applied in clinical routine to determine prognostic, a better diagnostic or indicate alternative chemotherapy to brain tumor patient treatment.

Nomenclature

AT/RT	Atypical Teratoid/Rhabdoid Tumors
BRAF	v-raf murine sarcoma viral oncogene homolog B1
CDK6	cyclin-dependent kinase 6
CDKN2A	cyclin-dependent kinase inhibitor 2A
CDKN2A/p16INKa	cyclin-dependent kinase inhibitor 2A (encoding p16)
CDKN2B/p15ARF	cyclin-dependent kinase inhibitor 2B (encoding p15)
CGH	Comparative Genomic Hybridization
CIN	Chromosomal Instability
CML	Chronic Myelogenous Leukemia
CNS	Central Nervous System
DM	Double-minutes
EGFR	Epidermal Growth Factor Receptor
ETANTR	Embryonal Tumors with Abundant Neuropil and True Rosettes

ETMR	Embryonal Tumors with Multilayered Rosettes
FISH	Fluorescence in situ Hybridization
GBM	Glioblastomas
GEP	Gene Expression Profile
HER2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog
HSR	Homogeneously Staining Region
IDH1	isocitrate dehydrogenase 1
IDH2	isocitrate dehydrogenase 2
Ink4A/Arf	cyclin-dependent kinase inhibitor 2A
LOH	Loss of Heterozygosity
MB	Medulloblastoma
M-FISH	Multiplex-FISH
MGMT	O-6-methylguanine-DNA methyltransferase
MYC	v-myc myelocytomatosis viral oncogene homolog
MYCN	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived
NF-kB	Nuclear Factor kappa B
NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
NSCLC	Non-small Cell Lung Cancer
OTX2	orthodenticle homeobox 2
P14ARF	cyclin-dependent kinase inhibitor 2A (encoding p14)
PCR	Polymerase Chain Reaction
PNET	Primitive Neuroectodermal Tumors
PTEN	phosphatase and tensin homolog
SCNA	Somatic Copy Number Aberrations
SHH	Sonic Hedgehog
SKY	Spectral Karyotyping
SMARCB1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1.
TGF-β	transforming growth factor, beta
TP53	tumor protein p53
USP4	ubiquitin specific peptidase 4
WBRT	Whole Brain Radiation Therapy
WHO	World Health Organization

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