Oligodendrogliomas: Impact of molecular genetics on treatment

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The interest in oligodendrogliomas has increased since it became evident that a subset of these tumors respond to chemotherapy or radiation. This interest was augmented when the combined loss of the short arm of chromosome 1 and the long arm of chromosome 19 was identified as a powerful prediction factor for response. Lack of stringent morphological criteria allow high-interobserver variation with regard to classification and grading of oligodendrogial tumors. The prospect of beneficial chemotherapy prompted neuropathologists to diagnose more ‘oligodendroglioma’ than before. Therefore, there is great demand for unambiguous classification of oligodendroglial tumors. Supplementary analysis of the integrity of chromosomal arms 1p and 19q may greatly assist diagnostic characterization of tumors with oligodendroglial phenotype. The underlying mechanisms for these deletions are not known. Tumor suppressor genes on 1p and 19q relevant for oligodendroglioma have not yet been identified. Knowledge of these genes and the mechanisms of their inactivation might help to understand why oligodendroglial tumors do respond better to chemotherapy and radiotherapy than astrocytomas. This review compiles clinical, pathological and molecular genetic findings on oligodendrogliomas and oligoastrocytomas of WHO Grades II and III to present a brief overview on recent developments.

Key Words: Oligodendroglioma, oligoastrocytoma, pathology, proto-oncogene, suppressor gene, tumor

Neuropathology of oligodendroglial tumors

Morphology

The distribution between frontal, parietal, temporal, and occipital lobe approximates 3:2:2:1. Rarely, oligodendrogial tumors affect the cerebellum, brain stem and spinal cord. Oligodendrogliomas impose as soft, gelatinous grayish-pink colored masses with well-delineated borders. Calcification may appear as gritty texture in unfixed tissue. Hemorrhages are not uncommon in oligodendrogliomas. Necrosis occurs only in anaplastic oligodendrogliomas WHO Grade III.

Microscopicall, oligodendrogliomas WHO Grade II are moderately cellular, monomorphic gliomas with rounded nuclei [Figure 1A: H&E]. A characteristic artifact is clear cytoplasm (‘honeycomb’ appearance) following standard tis-
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Oligodendrogliomas are moderately cellular, monomorphic tumors with rounded nuclei and clear cytoplasm. Calcification is frequent. Typical are numerous delicate, branching vessels with a "chicken wire" or "retiform" appearance. Perineuronal satellitosis, subpial, and perivascular tumor cell accumulation are frequent findings. GFAP-positive minigemistocytes can be found. Diagnostic criteria for anaplasia are ill defined. Cell density is either focally or diffusely increased. Cytology descriptions of the tumor cells range from atypical to frankly pleomorphic cells or even multinucleated giant cells. Mitotic activity may be brisk. Intratumoral changes range from increased density of branching vessels to glomeruloid microvascular proliferation. Both geographic necrosis and necrosis with perinecrotic pseudopallisading may occur.

Oligoastrocytomas or mixed gliomas exhibit oligodendrocytic and astrocytic components. Immunohistochemical characteristics

There are no routine antibodies to positively identify oligodendrogliomas. Immunohistochemical examinations are useful for exclusion of other glioma entities. Specific proteins of mature and developing oligodendrocytes like myelin basic protein (MBP), galactocerebroside (GaLC), or Myelin-associated glycoprotein (MAG) are only inconsistently expressed in oligodendrogial tumor cells. Recently, high-transcriptional activity of the oligodendrocyte lineage genes Olig-1 and Olig-2 encoding transcription factors expressed in early developmental and mature stages of oligodendrocytes was suggested for oligodendrogliomas, but not for astrocytomas by in situ hybridization. Meanwhile, multiple studies showed the expression of these transcription factors in both entities. Other oligodendrocytic lineage markers like PDGF-R, PLP and NG2 failed to specifically label oligodendrogliomas. Antibodies targeting glial fibrillary acid protein (GFAP) usually do not bind to oligodendroglial cells. However, so-called "mini gemistocytes" seen in a fraction of oligodendrogliomas strongly express GFAP. The antibody Mib1 that binds to Ki67 antigen is very useful in the assessment of proliferation. In many cases, less than 5% of tumor cells in oligodendroglioma WHO Grade II express Ki67.

Therapy of oligodendrogial tumors

Surgery

The traditional treatment of oligodendroglioma was of gross total resection. This approach decompresses mass effect and prolongs survival. In addition, surgical specimens allow neuropathological evaluation with classification and grading of the tumor.

In most series, the outcome of patients with low-grade oligodendroglioma correlates with the extent of surgery. However, one study found no difference in outcome between partial and gross total resection. This notion may be supported by a recent study on patients with anaplastic oligodendroglioma treated with PCV (Procarbazine-Vincristine-CCNU) chemotherapy with no difference in outcome regardless of partial or gross total resection or biopsy only approach.

Radiation therapy

Post surgery radiation with 45–65 Gy is a common stand-
ard for anaplastic oligodendroglioma WHO Grade III. Most authors found a clear benefit from radiation therapy. On the other hand, some investigators failed to demonstrate a significant effect of radiation on survival rate. These differences may be due to the inclusion of patients with oligodendrogliomas regardless of histological tumor grading. In oligodendrogial tumors WHO Grade II, no major benefit by radiation therapy was reported by most studies. However, a few series detected a moderate benefit for patients, particularly in cases with subtotal surgical resection. It should be kept in mind that patients with oligodendroglial tumors do have a life expectancy of several years warranting great care to avoid radiation-induced lesions such as leukencephalopathy. Therefore, it was recommended to delay radiation therapy in low-grade oligodendrogliomas until anaplastic transformation or tumor recurrence occurs.

**Chemotherapy**

In 1988, Cairncross et al. reported the response of anaplastic oligodendroglioma to chemotherapy. Later, PCV was identified as the most effective combination. Approximately 75% of patients with anaplastic oligodendroglioma responded to PCV while 25% of patients did not benefit from this treatment. Oligoastrocytomas seem to respond to PCV therapy, too. Meanwhile, chemotherapy with PCV prior to irradiation therapy has been recommended for newly diagnosed and recurrent oligodendroglioma. A novel drug introduced for treatment is Temozolomide, generally well tolerated and not associated with cumulative myelosuppression. However, up to now no phase III study compared the outcome of patients randomized for PCV or Temozolomide treatment. Recently, it was recommended to use Temozolomide as the last option in patients with recurrent anaplastic oligodendrogliomas. A recent report suggested treatment with Temozolomide for patients with oligodendroglioma WHO Grade II.

Successful chemotherapy and prolonged survival are associated with an increased incidence of secondary leukemia. Up to now, no clinical or morphological features are known for identification of patients likely to respond to chemotherapy.

**Prognosis of oligodendrogial tumors**

The prognosis of patients with oligodendrogial tumors varies due to different inclusion criteria for patients. The median postoperative survival time of oligodendrogliomas WHO Grade II ranged from 3.5 to 16.7 years. The 5-year survival rate varies between 38 and 83%. Progression to anaplasia does occur; but at lower frequency than in astrocytomas.

The median postoperative survival of anaplastic oligodendrogliomas WHO Grade III ranged from 0.9 to 7.3 years. The 5-year survival rate ranged from 23 to 66%. Chemotherapy of anaplastic oligodendrogliomas has prolonged the median time to progression to 25 months for responders. The largest series reported that 50 of 93 patients with anaplastic oligodendrogliomas treated either by chemotherapy or radiation showed tumor progression after a median of 48 months.

Only few data are available for low-grade oligoastrocytomas. The median postoperative survival times ranged from 3.9 to 6.3 years with a 5-year survival rate of 58%. One study reported a median time of survival in anaplastic oligoastrocytomas similar to anaplastic astrocytomas and less of that in anaplastic oligodendrogliomas.

Among the most exciting observations in oligodendrogliomas was the recognition of an association of allelic losses [Table 1] on chromosomal arms 1p and 19q and good response to chemotherapy. In a series of 39 patients with anaplastic oligodendroglioma, nearly all of those 70% with positive response to PCV chemotherapy exhibited loss of heterozygosity (LOH) 1p/19q. A significant association between a poor outcome and homozygous loss of CDKN2Ap16 was observed.

Another study confirmed the association of LOH 1p/19q and prolonged overall survival in oligodendrogliomas WHO Grade II. No survival advantage for patients with oligoastrocytomas (19 cases) and glioblastomas with LOH 1p/19q were seen. In contrast, two studies with low number of cases reported a positive association between LOH 1p/1q9 and response to chemotherapy in oligoastrocytomas or oligoastrocytomas and glioblastomas.

Oligodendrogliomas WHO Grade II can be separated in tumors with a more classical oligodendrogial and in tumors with a more astrocytic histological appearance. Indeed, genetic analyses demonstrated 1p loss in 19 of 22 classic oligodendrogliomas (86%) and maintenance of both 1p alleles in 16 of 22 gliomas with astrocytic features (73%). Interestingly, the predictive quality regarding response to chemotherapy was lower for the two histologically defined groups than genetic analysis of LOH 1p. Later, another study reported similar results by correlating histological features with the 1p/19q genotype and demonstrated an association between anaplastic features and loss of 9p or CDKN2A alterations.

Further, LOH 1p was shown to be a predictor of progression-free survival for patients with oligodendrogliomas WHO Grades II and III that received both, PCV chemotherapy and radiotherapy. Recently published data indicate a similar outcome for patients with 1p/19q LOH that were treated at the time of diagnosis with both PCV chemotherapy and radiotherapy, or only with chemotherapy. To avoid neurotoxic side effects, the authors suggested delaying radiation therapy until tumor recurrence. Other studies support this positive correlation between LOH 1p/19q and survival. In general, it was recommended to use PCV chemotherapy for...
patients with oligodendrogliomas exhibiting LOH 1p and to use radiation treatment for patients that retain both copies of chromosome 1p.\textsuperscript{[78]} Negative molecular predictors for overall survival in anaplastic oligodendrogliomas were found to be CDKN2Ap16 deletions and LOH 10q.\textsuperscript{[66],[79],[80]} In fact, these genetic alterations are typically seen in glioblastoma. However, only small groups of patients were analyzed.

**Molecular pathology of oligodendroglial tumors**

**Allelic loss of chromosome 1p and 19q**

The hallmark of oligodendrogliomas WHO Grade II is the combined allelic losses on the short arm of chromosome 1 (1p) and on the long arm of 19 (19q). These chromosomal alterations [Figure 2] were found in up to 90% of oligodendrogliomas.\textsuperscript{[81]–[85]} The high frequency of losses raises the possibility classifying oligodendrogliomas WHO Grade II by molecular alterations rather than by morphology. In anaplastic oligodendroglioma WHO Grade III, the frequency of LOH 1p/19q is lower. This might indicate either a broader spectrum of genetic alterations that induces these tumors or point toward more difficulties distinguishing anaplastic gliomas.\textsuperscript{[86]} Usually a combined loss of both chromosomal arms is observed but LOH 1p alone is also seen in oligodendroglial tumors.\textsuperscript{[86]} On the other hand, LOH 19q alone is a chromosomal alteration associated with the progression of astrocytic tumors.\textsuperscript{[87]} No sequential pattern of losses can be seen (unpublished own data).

Recently, an association between 1p/19q LOH and extra-temporal location of anaplastic oligodendroglioma has been described. Tumors that retained both alleles predominantly localize to the temporal lobe. Bilateral growth of anaplastic oligodendrogliomas was linked to LOH 1p/19q.\textsuperscript{[88]} In a larger series of tumors consisting of oligodendrogliomas and Oligoastrocytomas, a similar distribution was identified. In contrast, TP53 mutations were seen mostly in temporal oligoastrocytomas but not in extra-temporal tumors. In astrocytic tumors, no link between mutational spectrum and location was observed.\textsuperscript{[89]}

Classical tumor suppressor genes map to chromosomal regions with frequent allelic losses.\textsuperscript{[90]} Therefore, multiple deletions studies on both chromosomal locations were performed and candidate regions were identified. Deletion mapping of candidate regions on chromosome 1p was performed on those rare oligodendrogliomas with interstitial deletions. Three different candidate regions were identified on 1p, indicating the involvement of multiple tumor suppressor genes in oligodendrogliomas: 1p36.3 [Figure 3, I], 1p36.1–2 [Figure 3, II] and 1p34-35 [Figure 3, III]. [74, 91-96] Candidate genes like TP73 (1p36.3), CDKN2C (p18INK4c, 1p32), and hRAD54 (1p32) already have been excluded as major tumor suppressor gene candidates.\textsuperscript{[91],[92]–[97]}

Deletion studies face the problem of complete loss of 19q in oligodendroglioma.

Therefore, most fine mapping studies were based on astrocytic tumors, which frequently exhibit interstitial deletions on 19q.\textsuperscript{[85],[98]–[100]} Initially a common region of overlap was found between 19q13.11 and 19q13.4,\textsuperscript{[85]} which consecutively was narrowed down to 150 kb of genomic sequence.\textsuperscript{[98],[101]–[104]} However, none of the genes within these 150 kb, GLTSCR1, EDH, GLTSR2, and SW, carried mutations or were obviously deregulated.\textsuperscript{[105],[106]} Therefore, based on meta-analysis of multiple studies and employing physical sequence data from the human genome project, the candidate region was re-expanded to 3.7 Mb between D19S219 and D19S112.\textsuperscript{[107]} Up to now, several attractive tumor suppressor

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**Figure 2:** Chromosomal and genetic alterations in oligodendrogliomas. Chrom. Alt.: chromosomal alterations, LOH: loss of heterozygosity, Amp.: amplification. For further details see the text.

**Figure 3:** Mapping results for tumor suppressor gene identification on 1p and 19. For further details see the text.
genes like $RAX$, $CRX$, or $STD$ have been screened for mutations but no genetic alterations were identified.\textsuperscript{[107]} Alternative mechanisms of inactivation have been suggested. \textsuperscript{[107]–[109] Hypermethylation as a potential mechanism of gene inactivation appears possible: the density of CpG islands within the 19q candidate region is higher than at any other genomic site.\textsuperscript{[107]} This theory has become more attractive by observation of exclusive losses of the paternal allele in oligodendrogliomas (six of six cases), suggesting germ-line imprinting of the maternal copy of the gene.\textsuperscript{[110]} However, two follow-up studies based on larger sets of samples clearly showed an equal distribution of losses of parental alleles.\textsuperscript{[111],[112]}

Other chromosomal alterations were detected at a lower frequency. LOH studies, CGH, and karyotyping identified chromosomes 4, 6, 11p, 14q, and 22q as potential targets.\textsuperscript{[84],[113],[115], [117]} LOH 14q was observed in up to 30% of two candidate regions and an association with LOH 1p was identified.\textsuperscript{[117]} So far, for the other locations neither deletion mapping was performed nor associations with LOH1p/19q have been reported. LOH 17p and TP53 mutations were found rarely in oligodendrogliomas WHO Grade II with a frequency of 10–15% and usually inversely associated with LOH 1p/19q.\textsuperscript{[89],[118]–[122]}

However, epigenetic silencing of the $p14ARF$ gene that stabilizes the p53/MDM2 complex suggests a disruption of the p53 pathway in up to 20% of analyzed cases.\textsuperscript{[119],[121]}

\textbf{Progression-associated alterations in oligodendrogliomas}

Progression-associated alterations similar to those seen in malignant astrocytomas can be found in anaplastic oligodendrogliomas. Multiple genes in the RB1 pathway are altered: CDKN2A on 9p21 was identified as a major target of homozygous deletions in anaplastic oligodendrogliomas.\textsuperscript{[80],[79],[115],[123],[124]} Usually, these deletions include CDKN2B and the splice variant p14ARF\textsuperscript{[124]} In addition, these genes are frequently silenced by promoter hypermethylation.\textsuperscript{[121],[124]} Further, CDK4 amplifications and RB1 alterations were described.\textsuperscript{[124]}

Deletions of chromosome 10 were found in up to 58% of anaplastic oligodendrogliomas.\textsuperscript{[80],[114],[125]} An association between allelic loss of 10q and a poorer outcome, similar to astrocytic tumors, was described.\textsuperscript{[80],[126],[127]} However, another study failed to detect such association.\textsuperscript{[80]} This may point to the diagnostical problem of distinguishing anaplastic oligoastrocytoma from glioblastoma typically exhibiting LOH 10. Frequently, an inverse association between LOH 10q and LOH 1p/19q was reported.\textsuperscript{[73],[75],[79],[120],[128]} The tumor suppressor gene $PTEN$ on 10q23.31 is mutated only in 3–10% of oligodendrogial tumors.\textsuperscript{[80],[128],[133]} Those patients with $PTEN$ mutations exhibited a poor outcome.\textsuperscript{[131]} However, the high frequency of LOH 10q, the low number of $PTEN$ mutations and observation of interstitial deletions on 10q25–q26 telomere to $PTEN$ indicate the existence of another, yet not identified, tumor suppressor gene on this chromosomal arm.\textsuperscript{[122]} Recently, point mutations in the oncogene PIK3CA on 3q26.3 that antagonizes $PTEN$ were described in 14% of anaplastic oligodendrogliomas WHO Grade III.\textsuperscript{[130]} Promoter Hypermethylation of $MGMT$ on 10q26.3 was found in 60–80% of oligodendrogliomas. Only in a few samples, amplification of $EGFR$ and PDGFRA was seen in anaplastic oligodendrogliomas.\textsuperscript{[79],[115],[134]–[137]}

\textbf{Promoter hypermethylation in oligodendrogliomas}

Epigenetic silencing may play an important role in the induction and progression of oligodendrogliomas.\textsuperscript{[80],[138]} In fact, a high throughput approach identified large numbers of hypermethylated genes. However, most of these genes do not map to the 1p and 19q candidate regions.\textsuperscript{[118]} It should be kept in mind that methylation studies in oligodendrogliomas suffer the problem of insufficient comparison methods: differences in methylation status are based on the comparison of oligodendrogliomas with normal brain tissue containing mixed cell populations. Specific genes were further characterized: CDKN2A, CDKN2B, $p14ARF$, $RB1$, $TP73$, $DAPK1$, $ESR1$, $GSTM1$, $THBS1$, $TIMP9$, HIC, and $MGMT$ were found to be epigenetically silenced.\textsuperscript{[119],[121],[124],[138],[143]} $p14ARF$, an alternative splice variant of CDKN2A, binds to Mdm2, thereby blocking complex formation of p53 with Mdm2, resulting in a reduced degradation of p53.\textsuperscript{[141]} $TP53$ mutations and $MDM2$ amplifications are rare in anaplastic oligodendrogliomas.\textsuperscript{[94],[121],[124],[145],[146]} But epigenetic silencing of $p14ARF$ was reported in up to 50%.\textsuperscript{[121],[122]}

Promoter Hypermethylation of $MGMT$ (O6-methylguanine-DNA methyltransferase) on 10q26.3 was found in 60–80% of oligodendrogliomas. An association with 1p/19q status and tumor grade has been described.\textsuperscript{[141],[144]}

\textbf{Oncogenes}

There is little information available on proto-oncogenes in human oligodendrogliomas. Activating mutations of onco genes are rare in these tumors. On the other hand, strong expression of receptor tyrosine kinases that activate PI3K/AKT, RAS/MAP-, and PLC/PKC-pathways are frequently encountered. Analysis of these tumor-promoting pathways are of major importance. $EGFR$ (epithelial growth factor receptor) is frequently amplified and over expressed in high-grade astrocytomas.\textsuperscript{[3]} In oligodendrogliomas, increased mRNA and protein expression was observed in up to 80%.\textsuperscript{[134],[115],[147],[154]} Similar expression levels for $EGFR$ were found in Grades II and III oligodendrogliomas, indicating that this alteration is an early event in tumorigenesis. However, in contrast to high-grade astrocytomas, most studies reported only $EGFR$ amplification in a few samples of anaplastic oligodendrog-
The ligand PDGF-AA (platelet-derived growth factor) is a major mitogen for oligodendrocyte O2A progenitors, indicating a role in oligodendrocyte differentiation. Increased expression of PDGF-A and the receptor PDGFR-α was found in up to 94% of Grades II and III oligodendrogliomas, suggesting antoacrine and paracrine stimulation. In 4 of 41 anaplastic oligodendrogliomas, PDGFR-α amplification were identified. However, another study failed to detect such mutations. Our own analysis for PDGFR-α point mutations in oligodendrogliomas failed to detect alterations. PTEN protein antagonizes the activity of phosphatidylinositol-3-kinases (P3k) that convert PIP2 to PIP3. PIP3 activates the oncprotein Akt that regulates other multiple proteins involved in cell cycle progression, cell growth and survival, cell migration, invasion, and angiogenesis. Recently, mutations in PIK3CA, a P3k family member, were observed in 3 of 21 (14%) anaplastic oligodendrogliomas WHO Grade III. Effects on O2A progenitor cells are also induced by other growth factors such as bFGF, IGF-1, TGF-α, CNF, NT-3, IL-2, and IL-6, which are frequently over expressed in oligodendrogliomas. Genetic alterations have not been described. Amplification of other oncogenes like CDK4 and MDM4 were observed only in a low frequency in anaplastic oligodendrogliomas. Recently, point mutations in the gene coding for the oncogenic PTEN antagonist PIK3CA have been described in 16% of anaplastic oligodendrogliomas WHO Grade III but no amplification of the gene was found.

Conclusion

No particular gene mutations have been identified in low-grade oligodendrogliomas so far. The putative tumor suppressors on 1p and 19q still await identification. This paucity of knowledge about mutations on the gene level contrasts that on astrocytoma with many well-established mutations. However, analysis of LOH 1p/19q in oligodendrogliomas is developing toward a valuable tool for therapeutic decisions in oligodendrogliomas while molecular examinations do not yet play a major role in the management of astrocytic tumors.

References


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