

## Workshop: Modern techniques for the pathological investigation of brain tumors

# Oligodendroglioma: Impact of molecular biology on its definition, diagnosis and management

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**Molecular genetics and biology have been having significant influence on the practice of neuro-oncology in recent years, with oligodendrogliomas being the most prominent example. The majority of oligodendrogliomas show remarkable sensitivity to chemotherapy, and the finding that the loss of chromosome 1p was tightly associated with the response opened a new era in which the treatment can be tailored for each tumor patient based on molecular genetic diagnosis. It has been noticed that histological features, when closely examined, are also correlated with the molecular genetic data, such as losses of 1p/19q/10q and TP53 mutation. Furthermore, expression profiling using microarray technology showed that oligodendrogliomas with 1p loss express high levels of neuronal genes, suggesting that the progenitor of those tumors may be shared with neurons. Considering the rapid progress of the knowledge in neural developmental biology, it is possible that the classification, definition and diagnosis of gliomas will be rewritten based on such knowledge at the molecular level in the near future, if they reflect the biological features of each tumor better than the histological diagnosis based mostly on morphology.**

**Key words:** chemosensitivity, molecular diagnosis, oligodendroglioma.

Oligodendroglioma has attracted increasing attention in recent years, after it was recognized as a special subset of diffuse gliomas that shows remarkable response to chemotherapy, and probably to radiotherapy as well. However, the current definition of oligodendrogliomas has a significant room for subjective judgement, thereby leading to inter-institutional variance on the diagnosis.<sup>1</sup> This is a serious

problem because discussion on the treatment outcomes of oligodendrogliomas would not be valid if based on ambiguous diagnosis. To provide optimal treatment for each patient, we should have a clear definition of oligodendrogliomas and accurate methods for the diagnosis. Here I would like to present a brief overview on how molecular genetics has emerged as a new modality to improve the diagnosis and management, and I will discuss a possible change in the way oligodendrogliomas are defined in the future.

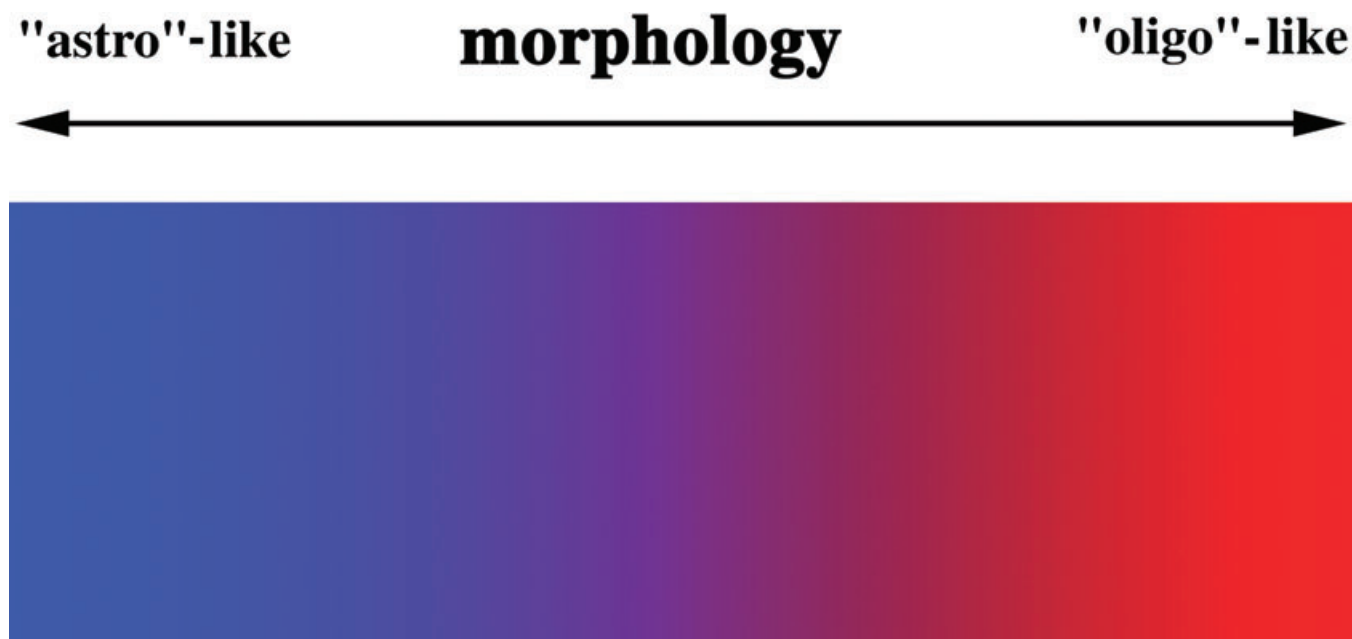
### HISTOLOGICAL FEATURES OF OLIGODENDROGLIOMAS

Oligodendrogliomas are composed of tumor cells with rounded, homogeneous nuclei accompanied with clear cytoplasm (perinuclear halo) on paraffin sections presenting so-called “fried egg” or “honeycomb” appearances. Dense networks of branching capillaries are also common, demonstrating the well-known “chicken-wire” pattern. Those histological features are easily recognizable in typical cases, making the diagnosis relatively straightforward. However, such typical patterns do not appear in peripheral areas where tumor cells infiltrate normal brain. Furthermore, the morphology of tumor cells often display variation even within a single case, showing more astrocytic features in some areas and oligodendroglial features in others. Among cases showing various degrees of mixed morphology, drawing a clear line between astrocytomas and oligodendrogliomas is difficult, and histological diagnosis becomes subjective. Because there is not a well-defined histological entity of oligoastrocytomas, many cases showing some histological variations can be put into this entity, and we occasionally see significant expansion of the range covered by this diagnosis by some pathologists. However, there has been a trend, especially in the USA, to make the diagnosis of oligodendrogliomas much more frequently, possibly influenced by psychological pressure from clinicians who are eager not to miss a favorable tumor. As a result, the number of cases diagnosed as oli-

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**Fig. 1** Morphological features of oligodendrogliomas and astrocytomas make a spectrum, with classic astrocytoma at one end and classic oligodendroglioma at the other.

godendrogliomas exceeded even that of astrocytic tumors in some institutions, although oligodendrogliomas used to represent only 10% or less of gliomas.<sup>2</sup> In order to organize such confusion in diagnosis, it would be helpful to schematize the situation as presented in Figure 1. This conceptual schema shows that the morphology of oligodendrogliomas and astrocytomas would make a spectrum, having typical oligodendrogliomas on one end and typical astrocytomas on the other. Where to draw the lines separating oligodendrogliomas, oligoastrocytomas, and astrocytomas within this spectrum is difficult, and therefore tends to be arbitrary. Probably, the truth was that it did not really matter for neurosurgeons or neuro-oncologists whatever the diagnosis was as long as it was within this spectrum, because the treatment was not much different anyway. However, this has changed significantly over the last two decades.

### **OLIGODENDROGLIOMAS AS A CHEMOSENSITIVE TUMOR**

In 1988, Cairncross and Macdonald reported that anaplastic oligodendrogliomas (World Health Organization (WHO) grade III) showed remarkable response to a chemotherapy regimen using procarbazine, CCNU, and vincristin (PCV therapy).<sup>3</sup> Those tumors often showed complete response to the chemotherapy administered alone, even at recurrence from successful initial treatment including radiation therapy. This turned out to be true for WHO grade II oligodendrogliomas, and oligodendro-

glioma has since been recognized as one of the most chemosensitive solid tumors.<sup>4,5</sup> In addition, it was, of course, good news for a patient that his/her tumor was likely to predict a better prognosis. It would not be surprising that such a situation has led to the increased diagnosis of oligodendrogliomas, as described earlier.

However, it was also true that not all oligodendrogliomas responded to chemotherapy; the response rate was in the range of 40–70%, and the next question to be asked was: which oligodendrogliomas were the good ones responding to chemotherapy, and how could we tell that?

### **MOLECULAR GENETIC FEATURES OF OLIGODENDROGLIOMAS**

Molecular genetic analysis on brain tumors was started on schwannomas by Seizinger *et al.* in the late 1980s, and was then applied to gliomas.<sup>6</sup> Since then, a flood of knowledge has accumulated over the last two decades, and gene mutations, gene amplifications, and chromosomal losses of various locations have been identified. Among those were mutations of *TP53* (*p53*), *CDKN2* (*p16ARF*), *PTEN*, gene amplifications of *EGFR* and *MDM2*, and chromosomal losses of 10q, 1p, 19q, 11p, and 22q. Those genetic alterations were found in various incidences in various grades/histological subtypes of gliomas. However, how those genetic alterations would affect the clinical practice was not clear at the beginning. In 1993, von Deimling *et al.* reported that, in glioblastomas,

there was a mutual exclusiveness between *TP53* mutation/17p loss and *EGFR* amplification, thereby dividing the glioblastomas into two separate subsets based on molecular genetic analysis. An important finding was that tumors with *TP53* mutations tended to occur in a younger population who fared better, whereas tumors with *EGFR* amplification were found in older patients whose prognosis was worse, in general.<sup>7</sup> This notion was soon confirmed by other groups as well, and it was also demonstrated that so-called primary glioblastomas (glioblastomas at the initial diagnosis) were more likely to harbor *EGFR* amplifications, whereas secondary glioblastomas (glioblastomas as a result of progression from lower grade gliomas) more commonly harbor *TP53* mutations.<sup>8</sup> This was the first example of the notion that molecular genetic analysis in neuro-oncology could provide clinically relevant information which histological diagnosis alone could not. With time, various positive and negative associations among genetic alterations were found in glioblastomas, and the current textbook of WHO classification on CNS tumors has integrated this knowledge, presenting genetic subsets of glioblastomas in a neat figure. The idea of genetic subsets, then, may be applicable to oligodendrogliomas.

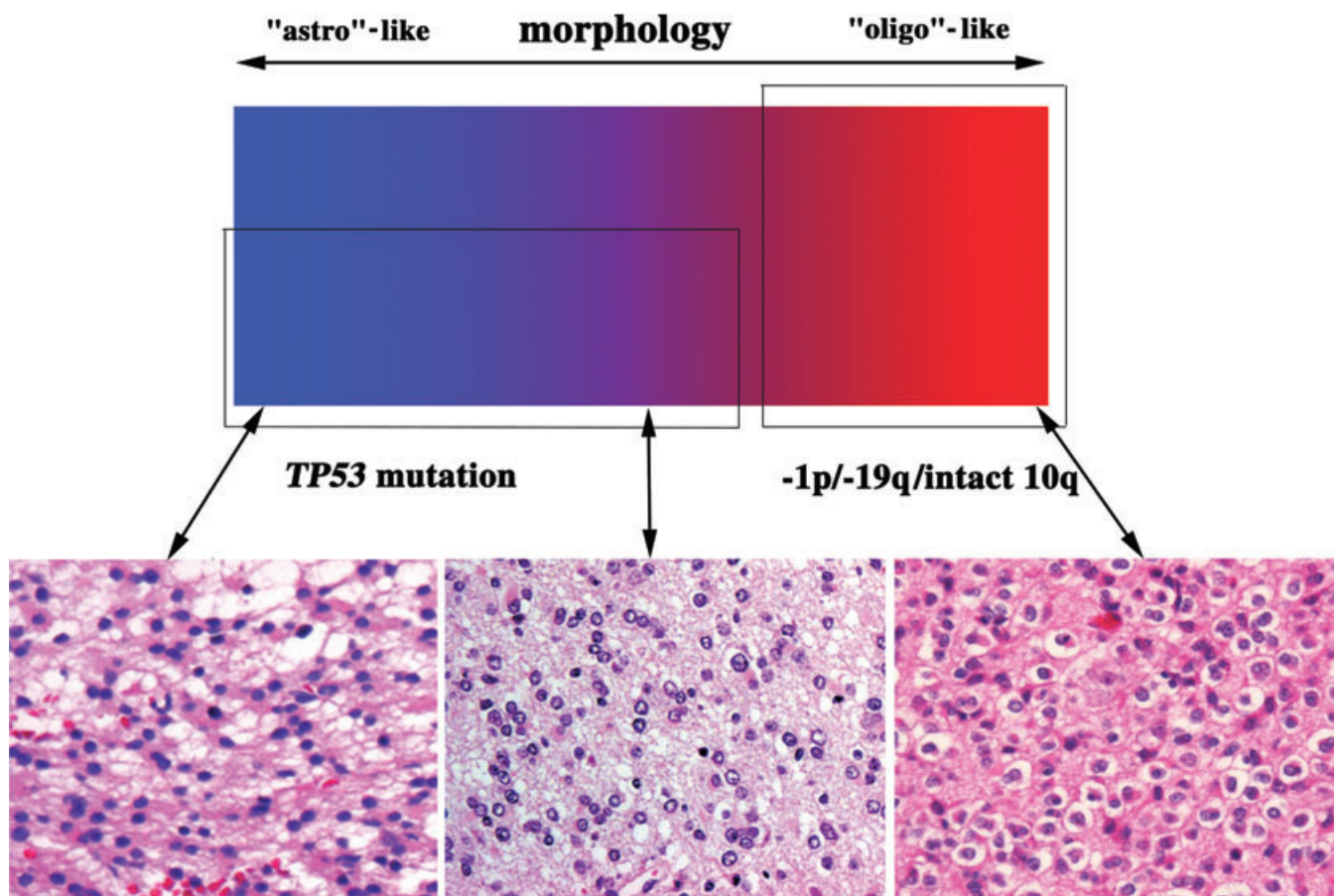
In oligodendrogliomas, it has been known that losses of chromosome 1p and 19q were frequently observed, up to 40–70%, and those two genetic alterations most commonly appear simultaneously in oligodendrogliomas.<sup>9</sup> In contrast, the incidence of *TP53* mutations was not as frequent as in astrocytomas, remaining within the range of 10–20%. However, it was not until 1998 that those genetic alterations were linked to the clinical outcome, when Cairncross *et al.* analyzed a series of anaplastic oligodendrogliomas treated by PCV chemotherapy as a major modality. Their study found that losses of chromosome 1p and 19q, especially the former, was closely associated with the chemosensitivity of oligodendrogliomas.<sup>10</sup> Therefore, oligodendrogliomas can be divided into a better-prognosis subset and a worse-prognosis subset by chromosomal 1p loss. This was an important achievement in neuro-oncology in which they first demonstrated that molecular genetic analysis would influence the management of patients with gliomas. Several studies followed to confirm and supplement the notion.<sup>11</sup> For instance, it was noted that *TP53* mutations were almost exclusively found in oligodendrogliomas without 1p loss, and that loss of 10q indicated a prognosis similar to glioblastomas.<sup>12,13</sup> All of those results indicated that examination of chromosome 1p/19q loss was essential in glioma cases when oligodendroglioma was suspected. Then, many others started to ask further questions: what was oligodendroglioma, and what was the meaning of 1p/19q loss in gliomas in general?

## CORRELATION OF HISTOLOGY AND MOLECULAR GENETICS

To the eyes of experienced neuropathologists, there were noticeable differences in the histological features between oligodendrogliomas with 1p/19q loss and those without. Burger *et al.* examined the 1p/19q status of 18 gliomas which were referred to them as possible oligodendrogliomas, and found the combination of 1p/19q loss in six cases. After reviewing the sections, all cases with 1p/19q loss were confirmed to be oligodendrogliomas by their standard, whereas the remaining 12 cases were diagnosed as astrocytomas, oligoastrocytomas, or glioblastomas.<sup>14</sup> Sasaki *et al.* reviewed 44 cases of pure low-grade oligodendrogliomas, and divided those into two groups: classical oligodendrogliomas and oligodendrogliomas with more astrocytic features. 1p loss was found in 19 of 22 (86%) classical oligodendrogliomas, and 16 of 22 (73%) oligodendrogliomas with astrocytic features maintained both 1p alleles.<sup>15</sup> Ueki *et al.* performed a molecular genetic examination on 91 gliomas including 33 oligodendrogliomas and oligoastrocytomas, and compared the results with the histological diagnoses by four independent neuropathologists. There were eight cases unanimously diagnosed as pure oligodendroglioma or anaplastic oligodendrogliomas by all four neuropathologists, and all of those had 1p/19q loss except for one with non-informative 1p status. *TP53* mutation was detected much more frequently in cases with astrocytic features, and 1p loss and *TP53* mutations were mutually exclusive in general.<sup>12</sup> Those studies confirmed that, although not clear-cut, there were histological features closely associated with the 1p/19q status and *TP53* mutations. If histological variance in astrocytic and oligodendroglial tumors makes a spectrum as was shown in Figure 1, 1p/19q losers should aggregate at the oligodendroglial end, whereas *TP53* mutators mostly be located at the other end (Fig. 2). What is important here is that molecular genetic data are clear-cut; “digital” data compared to the “analog” nature of histological diagnosis and drawing lines is much easier when based on molecular analysis.

## INFLUENCE OF RECENT PROGRESS IN BIOLOGY

Developmental biology has been one of the most exciting fields in molecular cell biology in recent years, and neural developmental biology is certainly a part of it. Identification of neural stem cells made an epoch, followed by rapid discovery of serial molecular events leading to the formation of various cell types constituting the nervous system.<sup>16</sup> Considering that all glioma classification and nomenclature refer to presumed origins of the tumor, it would be reasonable to expect that those new findings may advance



**Fig. 2** 1p/19q losses and *TP53* mutation are mutually exclusive, thereby clearly dividing the spectrum. Classic oligodendrogliomas (right) have 1p/19q losses and classic astrocytomas (left) tend to harbor the *TP53* mutation. Arguable oligodendrogliomas (middle) occasionally harbor *TP53* mutations; how to classify these tumors is not well determined yet.

the knowledge of glioma biology, and could provide new markers for the glioma classification and diagnosis. In oligodendroglioma, the expression of *OLIG1/OLIG2*, helix-loop-helix transcription factors specifically expressed in cells of oligodendroglial lineage, was investigated as a potential molecular marker for oligodendrogliomas.<sup>17,18</sup> Despite the promising early reports, many laboratories demonstrated that the *OLIG1/2* genes were expressed in a wide range of gliomas including astrocytomas, and the initial excitement seems to have diminished.<sup>19-21</sup> However, such investigations are still in their early phase, and further studies along this line may well be able to identify new molecular markers for oligodendroglioma.

Novel technologies have been shedding a new light on glioma biology as well. The invention of microarray technology has enabled the examination of comprehensive gene expression in a relatively short period, which has been applied to investigate oligodendrogliomas. Mukasa *et al.* used the GeneChip (Affimetrix, Santa Clara, CA, USA) system to see the expression profile of more than 20 000 genes in gliomas including oligodendrogliomas, astrocy-

tomas and glioblastomas. Genes showing significantly lower expression in oligodendrogliomas with 1p/19q loss included numerous genes on chromosome 1p and 19q, most likely reflecting the loss of whole arms of 1p and 19q.<sup>22</sup> When they examined genes expressed significantly higher in oligodendrogliomas with 1p/19q loss, many such genes were also highly expressed in normal neurons, but not in oligodendrogliomas without 1p/19q loss or other gliomas.<sup>23</sup> In line with this observation, recent studies demonstrated that there are indeed common precursor cells differentiating both into neurons and oligodendroglia, and histological observations also revealed that there are cases of oligodendrogliomas showing partial differentiation to neuronal cells.<sup>24</sup> Those data suggest the possibility of new markers for oligodendrogliomas with 1p/19 loss related to neuronal cells.

Investigating the origin of gliomas and the mechanism of tumorigenesis in humans is not an easy task because we can almost never follow the time-course of the initial tumor formation in patients. For such purposes, animal models reproducing the glioma formation process should

be one of the best tools. For example, Dai *et al.* created a mouse brain tumor histologically compatible with oligodendroglioma by overexpressing the *PDGF* gene in nestin-expressing neural progenitor cells, suggesting that those cells could be the origin of oligodendrogliomas.<sup>25</sup> At the same time, however, they also indicated that GFAP-positive astrocytes could also give rise to oligodendroglial tumors in the mouse when *PDGF* was overexpressed, leaving the conclusion on the origin of oligodendrogliomas still unsettled. Although many questions remain to be answered, similar efforts in the near future may resolve, at least in the mouse, which cells are the most likely origin of gliomas.

Another recent progress in cancer research related to developmental biology is the emergence of cancer stem cell theory. This theory proposes that cancer cells showing heterogeneous characteristics are born through asymmetric division of cancer stem cells, a small fraction of cells within the tumor which reproduce unlimitedly, like stem cells in normal development. A few laboratories reported isolation of glioma stem cells from human gliomas, suggesting that this theory might hold in gliomas as well. However, so far, such glioma stem cells are isolated only from highly malignant tumors, and whether low-grade gliomas also contain similar components remains to be investigated.<sup>26,27</sup> An interesting question would be whether such glioma stem cells, if they exist, arise from neural stem cells or through de-differentiation of more differentiated cells like astrocytes and oligodendrocytes. Animal models, again, would be the most promising tool to answer this important question.

### LOCATION–GENETIC CORRELATION IN OLIGODENDROGLIOMAS

There was one more surprising observation related to oligodendroglioma: the close association between location and genetic profile. When Zlatescu *et al.* compared the location of their series of oligodendrogliomas with genetic analysis results, they noticed that tumors with 1p/19q loss, which were practically 100% chemosensitive, were mostly located in the frontal, parietal and occipital lobes, whereas tumors with intact 1p/19q were found in the temporal lobe, insula and diencephalon. Statistic significance ( $P < 0.001$ ) was unequivocal.<sup>28</sup> There seems to be two viewpoints from which this unexpected finding could be explained. One is from the environment: the specific environment in each location may provide a growth advantage to a specific genetic alteration. The other is from the tumor origin: there may be two groups of precursor cells, one giving rise to oligodendroglioma with 1p/19q loss and the other to the tumors without, and those precursor cells are distributed unevenly within the brain. Each of the two explanations

would be possible, but I would prefer the latter considering the expression profiling data suggesting the different precursor cells. Either way, further studies to solve this mystery are awaited, which should shed new light on oligodendroglioma biology.

### MECHANISM OF CHEMOSENSITIVITY: ANOTHER UNSOLVED QUESTION

An important question is what is the molecular basis for the chemosensitivity in 1p/19q-losing oligodendrogliomas? If this question is answered, that could be extrapolated to other types of gliomas and greatly advance the knowledge on glioma chemosensitivity. Expression of *MGMT* was proposed to be a major factor associated with the chemosensitivity in gliomas, and comparison between *MGMT* expression and 1p/19q status in oligodendrogliomas found some positive association.<sup>29,30</sup> At this point, however, no specific molecules or genes mapped to 1p or 19q are linked to the chemosensitivity observed in 1p/19q losing oligodendrogliomas. Microarray studies did not come up with specific suspects possibly affecting the chemosensitivity either. Therefore, 1p/19q should be understood as a marker at this point, rather than the chromosomal location of genes involved in the chemosensitivity. We need further advancement in this front as well to improve patient care.

### TOWARD A BETTER DEFINITION OF OLIGODENDROGLIOMAS

With all those new findings accumulating, it seems to be a good time to start considering what is the best way to define and diagnose oligodendrogliomas.

Good definition and classification should have several features: (i) they should be based on feasible and simple methods; (ii) they should correlate well with clinical characteristics; and (iii) they should be objective to minimize inter-institutional variance. Histological diagnosis has maintained all those features well over the last several decades, and it is unlikely that molecular diagnosis of the current standard would replace it completely, although it may in the future. In the management of oligodendrogliomas, however, many neuro-oncologists now consider that examination of 1p/19q status is almost a “must” to provide optimal treatment for each patient if oligodendroglioma is suspected. Objectiveness is the obvious strength of molecular diagnosis, and the clinical course, such as chemosensitivity and prognosis, seems to be well predicted by molecular genetics. Furthermore, these molecular genetic tests now can be done in a relatively small laboratory in a short period, even more quickly than histological examination. Therefore, it would be reasonable to consider incorporating 1p/19q status and *TP53* mutation in the definition

of oligodendroglial tumors. For instance, it would be a possibility to use 1p/19q status to draw the line between oligodendroglioma and oligoastrocytoma. *TP53* mutation would be a strong indicator for astrocytoma, by contrast.

As for the general classification of gliomas, it may need to be rewritten along the context of neural developmental biology in the near future, in a way analogous to the classification of leukemia. Hemato-oncology is arguably the most successful field in oncology, and many leukemia patients are now practically cured by aggressive treatment. The introduction of potent chemotherapeutic agents and the advancement in bone marrow transplantation should be counted as the major factors for such success, but another factor was the establishment of a detailed classification based on the knowledge of hematopoiesis, identifying which stage of hematopoiesis the precursor cells giving rise to the specific subtype of leukemia were at the molecular level. Progress in neuro-oncology may be achieved through a similar path, and oligodendroglioma may well be a gateway to that goal.

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### REFERENCES

- Kleihues P, Louis DN, Scheithauer BW *et al.* The WHO classification of tumors of the nervous system. *J Neuropathol Exp Neurol* 2002; **61**: 215–225; discussion 226–219.
- Burger PC. What is an oligodendroglioma? *Brain Pathol* 2002; **12**: 257–259.
- Cairncross JG, Macdonald DR. Successful chemotherapy for recurrent malignant oligodendroglioma. *Ann Neurol* 1988; **23**: 360–364.
- Mason WP, Krol GS, DeAngelis LM. Low-grade oligodendroglioma responds to chemotherapy. *Neurology* 1996; **46**: 203–207.
- Olson JD, Riedel E, DeAngelis LM. Long-term outcome of low-grade oligodendroglioma and mixed glioma. *Neurology* 2000; **54**: 1442–1448.
- Seizinger BR, Martuza RL, Gusella JF. Loss of genes on chromosome 22 in tumorigenesis of human acoustic neuroma. *Nature* 1986; **322**: 644–647.
- von Deimling A, von Ammon K, Schoenfeld D, Wiesler OD, Seizinger BR, Louis DN. Subsets of glioblastoma multiforme defined by molecular genetic analysis. *Brain Pathol* 1993; **3**: 19–26.
- Watanabe K, Tachibana O, Sata K, Yonekawa Y, Kleihues P, Ohgaki H. Overexpression of the EGF receptor and p53 mutations are mutually exclusive in the evolution of primary and secondary glioblastomas. *Brain Pathol* 1996; **6**: 217–223; discussion 223–214.
- Kraus JA, Koopmann J, Kaskel P *et al.* Shared allelic losses on chromosomes 1p and 19q suggest a common origin of oligodendroglioma and oligoastrocytoma. *J Neuropathol Exp Neurol* 1995; **54**: 91–95.
- Cairncross JG, Ueki K, Zlatescu MC *et al.* Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. *J Natl Cancer Inst* 1998; **90**: 1473–1479.
- Smith JS, Perry A, Borell TJ *et al.* Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. *Clin Oncol* 2000; **18**: 636–645.
- Ueki K, Nishikawa R, Nakazato Y *et al.* Correlation of histology and molecular genetic analysis of 1p, 19q, 10q, TP53, EGFR, CDK4, and CDKN2A in 91 astrocytic and oligodendroglial tumors. *Clin Cancer Res* 2002; **8**: 196–201.
- Sasaki H, Zlatescu MC, Betensky RA, Ino Y, Cairncross JG, Louis DN. PTEN is a target of chromosome 10q loss in anaplastic oligodendrogliomas and PTEN alterations are associated with poor prognosis. *Am J Pathol* 2001; **159**: 359–367.
- Burger PC, Minn AY, Smith JS *et al.* Losses of chromosomal arms 1p and 19q in the diagnosis of oligodendroglioma. A study of paraffin-embedded sections. *Mod Pathol* 2001; **14**: 842–853.
- Sasaki H, Zlatescu MC, Betensky RA *et al.* Histopathological-molecular genetic correlations in referral pathologist-diagnosed low-grade “oligodendroglioma”. *J Neuropathol Exp Neurol* 2002; **61**: 58–63.
- Temple S. The development of neural stem cells. *Nature* 2001; **414**: 112–117.
- Marie Y, Sanson M, Mokhtari K *et al.* OLIG2 as a specific marker of oligodendroglial tumour cells. *Lancet* 2001; **358**: 298–300.
- Lu QR, Park JK, Noll E *et al.* Oligodendrocyte lineage genes (OLIG) as molecular markers for human glial brain tumors. *Proc Natl Acad Sci USA* 2001; **98**: 10851–10856.
- Riemenschneider MJ, Koy TH, Reifenberger G. Expression of oligodendrocyte lineage genes in oligodendroglial and astrocytic gliomas. *Acta Neuropathol (Berl)* 2004; **107**: 277–282.
- Bouvier C, Bartoli C, Aguirre-Cruz L *et al.* Shared oligodendrocyte lineage gene expression in gliomas and oligodendrocyte progenitor cells. *J Neurosurg* 2003; **99**: 344–350.
- Ligon KL, Alberta JA, Kho AT *et al.* The oligodendroglial lineage marker OLIG2 is universally expressed in

- diffuse gliomas. *J Neuropathol Exp Neurol* 2004; **63**: 499–509.
22. Mukasa A, Ueki K, Matsumoto S *et al*. Distinction in gene expression profiles of oligodendrogliomas with and without allelic loss of 1p. *Oncogene* 2002; **21**: 3961–3968.
  23. Mukasa A, Ueki K, Ge X *et al*. Selective expression of a subset of neuronal genes in oligodendroglioma with chromosome 1p loss. *Brain Pathol* 2004; **14**: 34–42.
  24. Perry A, Scheithauer BW, Macaulay RJ, Raffel C, Roth KA, Kros JM. Oligodendrogliomas with neurocytic differentiation. A report of 4 cases with diagnostic and histogenetic implications. *J Neuropathol Exp Neurol* 2002; **61**: 947–955.
  25. Dai C, Celestino JC, Okada Y, Louis DN, Fuller GN, Holland EC. PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes in vivo. *Genes Dev* 2001; **15**: 1913–1925.
  26. Singh SK, Hawkins C, Clarke ID *et al*. Identification of human brain tumour initiating cells. *Nature* 2004; **432**: 396–401.
  27. Yuan X, Curtin J, Xiong Y *et al*. Isolation of cancer stem cells from adult glioblastoma multiforme. *Oncogene* 2004; **23**: 9392–9400.
  28. Zlatescu MC, TehraniYazdi A, Sasaki H *et al*. Tumor location and growth pattern correlate with genetic signature in oligodendroglial neoplasms. *Cancer Res* 2001; **61**: 6713–6715.
  29. Mollemann M, Wolter M, Felsberg J, Collins VP, Reifenberger G. Frequent promoter hypermethylation and low expression of the MGMT gene in oligodendroglial tumors. *Int J Cancer* 2005; **113**: 379–385.
  30. Dong SM, Pang JC, Poon WS *et al*. Concurrent hypermethylation of multiple genes is associated with grade of oligodendroglial tumors. *J Neuropathol Exp Neurol* 2001; **60**: 808–816.