Dysembryoplastic neuroepithelial tumour: insight into the pathology and pathogenesis

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Abstract
Dysembryoplastic neuroepithelial tumour (DNT) is categorized as a benign glioneuronal neoplasm affecting children and young adults with chronic epileptic seizures. It is characterized by predominant intracortical localization and nodular architecture. Dysembryoplastic neuroepithelial tumour usually demonstrates a distinctive morphological pattern with a specific glioneuronal element but occasionally, its morphological picture is heterogeneous and unspecific. Thus, considering the morphology of DNT, three different histopathological subtypes are distinguished: simple, complex, and non-specific and diffuse. The DNT lesions are often related with focal cortical dysplasia (FCD) type IIb, which is postulated to play a role in epileptogenicity. Moreover, the accompanying inflammation process might be implicated in DNT-related epileptogenesis.

Dysembryoplastic neuroepithelial tumour is generally characterized by favourable prognosis and good results of surgical treatment. The pathogenesis and molecular mechanisms involved in DNT development remain uncertain. The main molecular findings are connected with BRAF alterations and activation of RAS/ERK, PI3K/AKT and mTOR signalling pathways. The present review summarizes the clinical, histopathological and molecular findings of DNT. The classification controversy, morphological heterogeneity and diagnostic problems are also discussed.

Key words: DNT, dysembryoplastic neuroepithelial tumour, histopathological variants, BRAF, mTOR, DNT – pathology and pathogenesis.

Introduction
Dysembryoplastic neuroepithelial tumour (DNT) is a benign, glioneuronal neoplasm that is included in the group of neuronal and mixed neuronal-glial tumours, according to the revised 4th edition of current ‘WHO Classification of Tumours of the Central Nervous System’ 2016 [46]. This slowly growing tumour corresponds histologically to WHO grade I [37]. Dysembryoplastic neuroepithelial tumour was first described in 1988 by Daumas-Duport et al. [17] as a distinct clinico-pathological entity associated with drug-resistant seizures, which affects children

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and young adults. Typically, the lesion is located in the cerebral cortex, particularly in the mesial temporal lobe. It exhibits multinodular architecture and appears in several different histopathological forms: 1) simple, 2) complex, 3) non-specific and diffuse. The most common clinical presentation is a drug-resistant epilepsy of a partial complex type, with early-onset and long duration from weeks to decades. The resection of the lesion is recommended in symptomatic cases. The stabilization of epileptic seizures has been demonstrated after total or even subtotal resection [12]. Occasionally, the recurrences and spontaneous or post radiation anaplastic transformation to high-grade gliomas have been noted [27].

**Localization**

Typically, the DNT lesion is located supratentorially in the cerebral cortex with a predilection for the mesial temporal lobe, less commonly for the frontal lobe [5,12,60]. It can be also found in other sites, including the septum pellucidum, caudate nucleus, brain stem and cerebellum [66]. Extremely rarely, the DNT located inside the ventricles, area of the corpus callosum and pericallosal region has been reported [2,11,23].

**Clinical presentation**

The main symptom of DNT is an early-onset, long-lasting, intractable epilepsy, mostly complex partial seizures with a frequent secondary generalization [5,12,41,59]. Seizures have their onset before the age of 20 years, usually in the childhood and continue for years to decades (mean duration of 10.8 years). The majority of cases are without focal neurologic deficits. Other symptoms, like dysarthria, facial asymmetry, focal weakness or numbness, visual field deficits or other visual symptoms are rarely encountered [16]. Moreover, the obstructive hydrocephalus could be seen in the septum pellucidum lesions [66].

**Neuroimaging findings**

Typically, DNT is a well-demarcated, multinodular lesion with predominant cerebral cortical location (Fig. 1A and B). It is hypointense on T1-weighted and hyperintense on T2-weighted magnetic resonance images (MRI) [35,36,59]. Dysembryoplastic neuroepithelial tumour is often composed of single or multiple cysts [59,67] and sometimes contains microcalcifications [16,59]. The deformations of the overlying part of the skull have been described. The oedema, mass

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Fig. 1. Magnetic resonance images (MRI) of dysembryoplastic neuroepithelial tumour (DNT). A) The multinodular intracortical lesion of high signal intensity on axial T2-weighted MR image. B) Coronal T2-weighted MRI with hyperintense cortical abnormality (Courtesy of Professor M. Roszkowski).
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effects on midline structures and contrast enhancement are not typical for DNT [36,67]. The internal separation and hyperintense “ring sign” at the periphery of the lesion might be revealed by MRI FLAIR sequence. The magnetic resonance spectroscopy (MRS) usually shows a low N-acetylaspartate peak and a lack of elevated choline-containing component (Cho) or Cho-Cr ratio (Cho/Cr) [67]. These features help to identify the DNT lesion and distinguish it from other low-grade gliomas.

**Histopathology**

Typically, DNT is characterized by intracortical nodules with columnar architecture of uniform oligodendrocyte-like cells within microcystic background (Fig. 2A and B). It exhibits a distinct morphology with a so-called specific glioneuronal element (Fig. 2C). This element consists predominantly of small oligodendrocyte-like cells arranged in a columnar orientation along parallel bundles of axons (Fig. 2D). They are usually oriented perpendicular to the cortical/pial surface [20]. Microcysts of various shape and size are lined by small, uniform cells of oligodendrocyte-like morphology (Fig. 2E and F). The large neurons are scattered within microcystic areas, sometimes making an impression that they “float” in mucin-filled background (Fig. 3A). The neurons usually exhibit morphology of normal pyramidal neurons (Fig. 3B) and may be considered as pre-existing cortical neuronal cells. Moreover, the various amount of astrocytes might be scattered in the DNT lesion. The above multinodular architecture of the lesion accompanied by the presence of “floating neurons” are the most distinctive morphological features of DNT.

Sometimes, the DNT may exhibit a growth pattern resembling diffuse gliomas. In some cases the DNT lesion consists of solid areas with oligodendrocyte-like cells and thin-walled microvessels (Fig. 3C). Delicate, so-called “chicken-wire” vasculature resembling oligodendroglioma can be visible (Fig. 3D). Microvascular proliferation with a telangiectatic pattern could be also seen. Some areas of DNT show hypercellularity with honeycomb appearance of monomorphic cells with round nuclei surrounded by clear haloes or ribbon-like palisades highly similar to oligodendroglioma (Fig. 3E and F). However, perinuclear satellitosis is not characteristic for DNTs lesions. Occasionally, the compact piloid tissue with pleomorphic neoplastic cells might resemble pilocytic astrocytoma.

Intriguingly, Komori et al. [33] claimed that DNT is rather a glial than glioneuronal tumour, similar to oligodendroglioma, thus it should be classified as a non-infiltrative oligodendroglioma. The research performed on DNT samples using morphometric evaluation and immunohistochemical studies documented staining for Neu-N, a neuronal nuclear antigen being a marker of neurons, and Olig-2, a transcription factor important for motoneuron [42,52] and oligodendrocyte differentiation [70]. They found that double immunohistochemical staining showed co-localization of Olig-2 and Neu-N. The distribution of Neu-N positive nuclei was similar in the tumour tissue and the adjacent cortex. Moreover, the density of Neu-N positive nuclei was lower in the tumours located in the white matter compared to those from grey matter. They suggest that probably Neu-N positive cells are entrapped in granular and pyramidal neuronal cells [33].

Three histopathological forms of DNT have been described, including: 1) simple, 2) complex and 3) non-specific and diffuse. The last form was created based on the statement that the diffuse form of DNT corresponds to a non-specific variant [8,19,29]. However, the diagnosis and definition of the non-specific type of DNT is controversial.

**The simple form of DNT** is composed of the specific glioneuronal element with oligodendrocyte-like cells arranged in columns. The bundles of axons and floating neurons are dispersed in a mucin-like background.

**The complex form of DNT** exhibits heterogeneous morphology with multinodular architecture. Except a specific glioneuronal element mentioned above, it is characterized by presence of glial nodules resembling oligodendrocytic or astrocytic population [18].

Diagnosis of a **non-specific and diffuse form of DNT** is challenging because of lack of the specific glioneuronal element and no multinodular structure. This form of DNT exhibits glial components similar to glial nodules of the complex DNT type. It is difficult to distinguish this DNT variant from other glial tumours. The non-specific and diffuse form of DNT might be histologically similar to glioma, ganglioglioma, pilocytic astrocytoma or diffuse astrocytoma [61,65]. Nevertheless, the clinical presentation with chronic seizures, cortical localization on neuroimaging and a follow-up are similar to typical DNT. Thus, the clinical data are mandatory to consider the identification of the non-specific and diffuse form of DNT [19].
Fig. 2. Histopathology of dysembryoplastic neuroepithelial tumour (DNT), H&E staining. A, B) Intracortical nodules with microcystic, vacuolated background. C) Specific glioneuronal component with typical columnar architecture. D) Microcystic region consisting of mucin-rich background and a small oligodendrocyte-like component arranged along parallel rows of axons. E) Microcystic architecture with uniform oligodendrocyte-like cells and tiny vessels. F) Large microcysts lined by small oligodendrocyte-like cells (from own archival surgical material).
Fig. 3. Histopathology of dysembryoplastic neuroepithelial tumour (DNT), H&E staining. A) Specific glioneuronal element with alveolar arrangement of oligodendrocyte-like cells and numerous interspersed floating neurons. B) Typical glioneuronal element with an easily visible floating neuron. C) Solid growth pattern with oligodendrocyte-like cells and small, thin-walled vessels. D) Delicate branching capillaries and small, uniform cells resembling oligodendroglioma. E) Diffuse growth pattern with honeycomb appearance mimicking oligodendroglioma. F) Small, monomorphic cells with round nuclei surrounded by clear haloes similar to oligodendroglioma (from own archival surgical material).
In accordance with the benign nature of DNT, cytological atypia or mitotic activity are uncommon features and Ki-67 proliferative index is usually low, ranges from 0 to about 1.6% [22].

Moreover, DNT may be associated with focal cortical dysplasia (FCD), hippocampal sclerosis and ganglioglioma [50,51,63]. Cortical dysplasia accompanying DNT lesions is identified as FCD type IIIb, according to the criteria of the classification of the International League Against Epilepsy [6]. The foci of cortical dysplasia often coexist with the simple and non-specific type of DNT [18,19].

Differences between various forms of DNT also manifest in epileptogenicity. Chassoux et al. [15], using stereo-electroencephalography, compared epileptogenicity in different histological variants of DNT. Interestingly, they have found that the epileptogenic zone is localized in the tumour in a simple and complex form and is more widespread in most non-specific DNTs. The difference is pronounced especially in non-specific, temporal DNTs with extensive focal cortical dysplasia (FCD). However, the relationship with FCD does not unambiguously explain this discrepancy.

In addition, rare mixed tumours composed of a DNT lesion with components of rosette forming glioneuronal tumour [38], pilocytic astrocytoma [44] or pleomorphic xanthoastrocytoma [30] have been described.

Immunophenotypic profile

Markers useful in characterization of immunophenotype of DNT include S-100 protein, transcriptional factor OLIG-2, glial fibrillary acid protein (GFAP), NeuN, microtubule-associated protein 2 (MAP-2), CD34, nestin, and calbindin.

The small oligodendrocyte cells express S-100 protein (Fig. 4A) and OLIG-2, whereas they are negative for GFAP. The neuronal component consisting of “floating neurons” shows neuronal markers such as NeuN, MAP-2 and synaptophysin. GFAP can be expressed in the astrocytes scattered in the background (Fig. 4B).

MAP2 protein was observed in different proportions among DNT variants. It is expressed during development of the central nervous system as well as in neurons and glial cells in the adult brain [58,64]. MAP2 expression in oligodendrocyte-like cells and glial elements was observed significantly more often in non-specific DNTs than in the simple or complex form. Co-expression of MAP2 and CD34 was significantly more frequent in the non-specific than in complex and simple form [62]. However, the difference in MAP2 expression among different types of DNT has not been confirmed [65].

A cell-surface transmembrane protein CD34 can be found in some cases of DNT. It is commonly used as a marker of hematopoietic stem, progenitor cells and vascular endothelial cells, which is associated with increased proliferation and abrogated cell differentiation [45]. CD34 staining was observed in the perikaryal membrane of neuronal cells, cytoplasm of oligodendrocyte-like cells, pericellular stroma and stellate cells with astroglial morphology. Irrespective of the classification to the non-specific or

![Fig. 4. Immunohistochemistry of dysembryoplastic neuroepithelial tumour (DNT). A) S-100 protein immunopositivity in oligodendrocyte-like elements. B) Glial fibrillary acid protein (GFAP) reactivity in the astrocytes scattered in the background (from own archival surgical material).](image-url)
diffuse form, CD34 expression in both types of DNT was found to be similar. Statistically significant differences of immunohistochemical staining for CD34 among three main histological types of DNT were observed in two studies. The highest expression of CD34 was noticed for the non-specific type compared with simple and complex types [62,65].

Expression of CD34 and nestin was found to be the highest in the non-specific and diffuse form, but there was a difference in expression of these markers in the simple type [61,62]. However, Thom et al. [65] observed a statistically significant correlation between CD34 and nestin staining in particular forms. Nestin is expressed during development of the central nervous system and in the majority of proliferating brain progenitor cells, thus it is considered as a neural stem cell marker [40]. Expression of nestin and CD34 was considerably higher in the non-specific and diffuse DNTs, which may suggest that it is a less differentiated form. Nevertheless, it is not related to higher malignancy [65].

Furthermore, the non-specific type and mixed DNT/GG tumours were characterized by a significantly higher expression of calbindin [65], a calcium binding protein expressed in neuronal cells [4].

**Molecular findings**

The main molecular findings are connected with BRAF alterations and activation of RAS/ERK, PI3K/AKT and mTOR signalling pathways.

BRAF is a RAF family member displaying the highest basal activity and being considered to play an important role in tumorigenesis [13,57]. It is implicated in the so-called mitogen-activated protein kinase (MAPK) pathway [39].

Chappé et al. [13] found BRAFV600E mutation in ca. 30% of DNT. Analysis was performed by a combination of polymerase chain reaction-high resolution melting (PCR-HRM), direct sequencing and immunohistochemistry as a complementary method.

![Fig. 5. Diagram illustrating probable mechanism leading to mammalian target of rapamycin (mTOR) activation. Activation of RAS/mitogen activated protein kinase (ERK) – p90 ribosomal six kinase (p90RSK) pathway as well as activating mutation of BRAFV600E may impact on separation of liver kinase B1 (LKB1) from AMP-activated protein kinase (AMPK), what leads to mTOR activation. However, the mechanism seems to be independent of phosphorylation of LKB1 by p90RSK.](image-url)
High occurrence of **BRAFV600E** mutations (51%) was detected in a study performed on a group of 51 DNT cases [34]. Furthermore, the authors have found that mutations were significantly more frequent in tumours with extratemporal location.

In another direct sequencing study performed on 77 samples of DNT, frequent **BRAFV600E** mutation was shown in approximately 30% of cases [47]. Intriguingly, the authors did not find **BRAFV600E** mutation in the simple form of DNT. They also reported immunohistochemical co-localization of **BRAF** V600E-mutated protein with phosphorylated ribosomal S6 protein (pS6) and phosphorylated liver kinase B1 (pLKB1), as well as they found a significant correlation between presence of **BRAFV600E** mutations and pS6 expression in dysplastic neurons in DNTs. The pS6 is a marker of mTOR activation. Thus, a mutation in **BRAF** might indirectly activate mTOR by the LKB1/AMP-activated protein kinase (AMPK) pathway (Fig. 5). LKB1 with its downstream effector AMPK may act as a tumour suppressor by downregulation of mTOR activity [26].

The crosstalk LKB1/AMPK with RAS/ERK/p90RSK pathway was previously described in melanoma cell cultures [25]. BRAF copy number gain was reported for the first time in DNT tumours by Kakkar et al. [32]. Alterations in the **BRAF** gene, and activation of mTOR and MAPK signalling pathways are suggested to play an important role in pathogenesis of DNT, and may be considered as a target for future treatment.

**Fig. 6.** Diagram illustrating activation of signalling pathways, which activation was examined in dysembryoplastic neuroepithelial tumour (DNT). Activation of the receptor, i.e. fibroblast growth factor receptor 1 (FGFR1) leads to RAS activation and consequently to phosphorylation of mitogen activated protein kinase (ERK) through cascade of protein kinases: RAF and mitogen activated protein kinase (MEK). Phosphorylated ERK may activate transcription factors as well as impact on tuberous sclerosis complex 2 (TSC2) – an inhibitor of mammalian target of rapamycin (mTOR). Lack of mTOR suppression implies cell growth and proliferation. Similarly to ERK, also phosphorylation of AKT by 3-phosphoinositide-dependent kinase 1 (PDK1) leads to mTOR activation. Phosphatase and tensin homolog (PTEN) might reverse phosphatidylinositol-3-kinase (PI3K) phosphorylation and inhibit activation of downstream proteins of the pathway.
The targeted therapies might be especially useful in patients with incomplete surgical resection and persistent seizures.

On the contrary, the study of Boer et al. [9] showed that activation of the mTOR pathway is similar to the control human brain. However, it should be stressed that this study was performed on nine samples of the simple DNT type, which may explain lack of active mTOR. Immunocytochemical analysis of activation of the PI3K/AKT pathway also revealed no differences as compared to the control brain.

Pathogenesis of DNT may also include phosphatase and tensin homolog (PTEN), a tumour suppressor protein, which is involved in regulation of the PI3K/AKT pathway [28] (Fig. 6). However, mutations of the PTEN gene were not detected using single strand conformation polymorphism (SSCP) analysis and direct sequencing in a sample of DNT [21]. Furthermore, it must be stressed that this investigation was performed on one DNT sample only.

The possible way of RAS/ERK, as well as PI3K/AKT activation, is phosphorylation of fibroblast growth factor receptor 1 (FGFR1). Investigations by Zhang et al. [69] on paediatric low grade gliomas showed duplication of the tyrosine kinase domain of FGFR1. It leads to autophosphorylation of the receptor and consequently RAS/ERK and PI3K/AKT upregulation.

Recent studies have shown genetic alterations of FGFR1 in 82% of examined DNTs, where dominant types of alterations were tyrosine kinase domain duplications and single nucleotide variations [53].

Furthermore, high frequency of FGFR1 mutations was presented by Rivera et al. [56]. Interestingly, they distinguished two groups of tumours from collected samples, which were primarily diagnosed as DNTs. Histopathological revision using current WHO diagnostic criteria distinguished the group of typical DNTs with characteristic glioneuronal elements. The non-DNT group was composed of the other tumours whose histopathological view and clinical features might correspond to the non-specific type of DNT. Mutations of FGFR1 were more frequent in specific DNT (58%) in comparison to the non-DNT group (19%). The main type of alteration in the specific DNT group was tyrosine kinase domain duplication. Moreover, mutation of BRAFV600E was observed in 22.6% of non-DNTs, while it was not detected in specific DNT.

In addition, the authors described germline FGFR1 mutation in the three familial cases with multino-
Immunohistochemical staining with specific marker as S-100, synaptophysin, CD34 or BRAF protein and analysis of genetic aberrations might be helpful to differentiate DNT from other brain tumours.

Evaluation of 1p/19q status as well as mutation of the IDH1 gene in codon 132 (R132H) may be useful to distinguish DNT from oligodendroglioma. Both, loss of heterozygosity for 1p, 17p and 19q [31, 49] and IDH1 gene mutation [10] are not identified in DNTs and their presence excludes the diagnosis of DNT lesion. Also, the IDH1 and TP53 mutation is helpful in identification of diffuse astrocytomas and distinguish them from DNT. Moreover, strong GFAP immunopositivity of neoplastic cells is related with astrocytic differentiation in pure astroglial tumours. The low proliferative index Ki67 might indicate the benign DNT tumour rather than diffuse gliomas.

Treatment and outcome

The predominant and the most effective treatment in DNT symptomatic cases is a total tumour resection. It leads to a seizure-free outcome even in more than 80% of patients during at least a one-year observation [12]. Even better results may be obtained by the complete resection of the tumour together with the epileptogenic zone [15]. Thus, it is believed that the majority of DNT lesions are surgically curable.

However, the cases of DNT with recurrences and malignant transformations of initially recognized benign tumours have been sporadically reported [1,27,68]. The malignant transformation of DNT seems to be unique, however it argues the possibility of more aggressive behaviour of this originally described benign lesion [6]. Time of recurrence after the initial resection may ranges between several months to several years. Occasionally, another brain tumour with higher malignancy has been detected after DNT resection, including atypical teratoid rhabdoid tumour (AT/RT), a highly aggressive embryonal brain tumour [43]. This emphasizes the importance of the prolonged period of clinical and neuroimaging follow-up of DNT resected lesions.

Prognosis

Most commonly, the DNT resection results in stabilization of the clinical course, even after subtotal resection. The meta-analysis conducted on 910 patients with DNT or ganglioglioma showed that the seizure outcome depends on the duration and type of epilepsy, and extensiveness of resection [24]. Secondly, generalized seizures, longer than one-year epilepsy and subtotal lesionectomy were connected with a worse seizure outcome. It was documented that there were no significant differences in the seizure outcome between DNT and ganglioglioma.

Another analysis conducted on data of 78 patients operated on for DNT confirmed a statistically significant correlation between a shorter duration of epilepsy before the surgery and a more favourable outcome [14].

Furthermore, a study on paediatric patients with glioneuronal tumours (ganglioglioma and DNT) showed that a shorter duration of seizures was connected with a better cognitive functioning [54]. It might indicate a negative effect of epilepsy as well as antiepileptic drugs on cognitive development.

Another study revealed the presence of cells of the microglial/macrophage cell system and indicated a significant functional correlation between HLA-DR positive cells and the duration of epilepsy and also a preoperative seizure frequency in glioneuronal tumours (gangliogliomas and DNTs) [3]. Moreover, the connection between the presence of microglial cells and the seizure outcome after surgery was found. The presence of microglial cells was connected with an inflammatory process that might have a negative effect on the disease process. The authors pointed out that the duration of epilepsy had an impact on the occurrence of microglial cells and probably also on the inflammation, which developed during the neoplasm growth.

Final considerations

The theory that DNTs are only glial tumours, whereas the neuronal component consists of entrapped normal neurons, demands further investigations on a larger group, which will contain also the non-specific and diffuse type. On the one hand, the occurrence of copy number aberrations only in the cells with glial morphology is in accordance with the mentioned theory. But, on the other hand, the mutations in BRAF were found in neuronal cells. Moreover, the FISH analysis of copy number aberrations should be performed on a higher number of cases to ensure it.

Differences in immunophenotype of distinct types of DNT may suggest a distinct origin of the non-specific and diffuse type or might indicate that it is an earlier form of DNT, which might progress
into the simple or complex form. The diagnosis of non-specific and diffuse variant of DNT is still not obvious. Moreover, the changes in classification of tumours associated with long-term epilepsy are postulated. The idea is to categorize all three types of DNT into different groups of tumours [7]. The proposed classification is based on histopathological features as well as immunohistochemical labelling (CD34, MAP2) and testing for IDH1 mutations.

Histological categorization might be supported by differences in molecular mechanisms involved in pathogenesis. BRAFV600E mutations seem to be implicated mainly in non-specific and diffuse types of DNT, while FGFR1 mutations were observed mostly in specific DNTs.

Moreover, the microglia cells and released cytokines seem to be implicated in the DNT pathological process and they are related to the clinical presentation. Probably, the role of microglia is associated with IL-1β release and induction of the inflammatory process, which causes seizures.

Summarizing, DNT is a benign tumour lesion of a distinct clinical and neuroimaging picture but heterogeneous, often confusing morphology, uncertain histogenesis and not fully defined molecular background.

Disclosure

Authors report no conflict of interest.

References


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