

Neuroblastoma update

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Purpose of review

The purpose of this paper is to review the most recent medical literature pertaining to neuroblastoma. Clinically, progress continues to be made on the refinement of radiographic staging and risk stratification-based treatment.

Recent findings

Meanwhile, experimental efforts focus on the continued search for biologic prognostic markers and the development of novel therapies including second-generation retinoids, tumor vaccines, and new modes of drug delivery with improved safety and efficacy.

Summary

Review of the recent neuroblastoma literature demonstrates continued progress in our ability to understand and treat this enigmatic tumor.

Keywords

neuroblastoma, risk stratification, retinoids, scintigraphy

Introduction

Review of the literature published during 2003 to 2004 reveals more than 1000 publications on neuroblastoma. From these we selected what we believed were the most important contributions and highlighted their findings in this update. We hope this update proves valuable in the ongoing quest to stay at the forefront of medical research and useful for treating patients with neuroblastoma.

Epidemiology

Because neuroblastoma mainly affects infants, studies have sought to identify an environmental exposure that could lead to disease. Breastfeeding has been reported as protecting against neuroblastoma [1]. A recent retrospective study examined the impact of a Canadian program of cereal fortification with folic acid on the incidence of neuroblastoma [2•]. This population-based study observed a decrease in incidence before and after folic acid fortification. Long-term studies and additional research into the role of folate metabolism in neuroblastoma are indicated, given these results.

Diagnosis and staging

Staging is an important part of evaluation of neuroblastoma and is based on the International Neuroblastoma Staging System (Table 1). Siegel *et al.* [3] evaluated the role of CT, MRI, and metaiodobenzylguanidine (MIBG) scintigraphy on staging stage 4 disease. They found that CT and MRI did not differ significantly, but the addition of scintigraphy to CT significantly improved the diagnosis in patients with stage 4 disease [3]. Similarly, Kushner *et al.* [4•] looked at the effect of MIBG scans on evaluating the effect of dose-intensive chemotherapy in high-risk patients. They found that MIBG scanning picked up bone marrow or cortical bone disease not otherwise discovered [4•]. These findings support the use of MIBG scintigraphy in patients with high-risk neuroblastoma.

The past year also marked the identification of a new phenotype of neuroblastoma with aggressive clinical behavior. The rare tumor, termed a large cell neuroblastoma, is a poorly differentiated Schwannian stroma-poor tumor composed of large cells with sharply outlined nuclear membranes and one to four prominent nucleoli. Because of its unique clinicopathologic features, it is proposed that large cell neuroblastoma be recognized as a distinct entity within the undifferentiated and poorly differentiated subtypes [5].

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Table 1. International neuroblastoma staging system

Stage	Description
1	Localized tumor. Complete excision, with or without microscopic residual. Ipsilateral lymph nodes negative.
2A	Localized unilateral tumor. Incomplete gross excision. Ipsilateral lymph nodes negative.
2B	Localized unilateral tumor. Complete or incomplete excision. Ipsilateral and regional lymph nodes positive. Contralateral lymph nodes negative.
3	Unresectable unilateral tumor infiltrating across the midline, with or without lymph node involvement. Unilateral tumor with contralateral lymph node involvement. Midline tumor with bilateral infiltration or bilateral lymph node involvement.
4	Dissemination of tumor to distant lymph nodes, bone, bone marrow, liver or other organs.
4S	Localized primary tumor in patients <1 year with limited dissemination to liver, skin or bone marrow.

Biologic markers

The continued investigation into prognostic genetic markers for neuroblastoma has been augmented by gene array analysis. Using this technology, a number of novel candidate prognostic markers for neuroblastoma have been identified including BIRC (associated with apoptosis), CDKN2D (associated with cell cycle), and SMARCD3 (associated with transcriptional activation) [6]. Similarly, expression profiling data have been used to elucidate genetic mechanisms behind telomerase activity in neuroblastoma cells. Specifically, genes involved with differentiation/growth arrest were closely related to low telomerase activity in neuroblastoma, whereas over-expression cell-cycle-related genes and transcriptional factors were associated with high telomerase activity [7]. Finally, full-length telomerase reverse transcriptase messenger RNA was found to be an independent prognostic factor in neuroblastoma [8].

Identification of anomalies of constitutional karyotype can lead to the discovery of oncogenes and tumor-suppressor genes. The most common abnormality in neuroblastoma is a gain at 17q. This genetic alteration is associated with adverse outcomes [9•]. Loss of heterozygosity at several sites has also been identified in neuroblastoma. Deletion of the short arm of chromosome 1 occurs in 30 to 50% of primary tumors, usually around 1p36, and strongly correlates with N-myc amplification and a poor prognosis [9•]. A recent Children's Cancer Group study showed that deletion at 1p was an independent predictor of decreased event-free survival, though not of overall survival [10]. Loss of heterozygosity at 11q and 14q has also been described. The deletion at 11q is identified in almost half of all neuroblastoma samples. Although inversely related to N-myc amplification, loss at 11q is associated with a worse prognosis [9•]. This year, new cases of neuroblastoma with other chromosomal abnormalities were reported, including a mosaicism for monosomy 22, 11q interstitial deletion, and a Robertson translocation t(13;14) [11•].

Risk stratification and treatment

Treatment of neuroblastoma is based on risk stratification categories. Patients with low-risk disease usually can be treated with surgery alone. Those patients with stage I disease have survival rates of more than 95% after surgery alone. Patients with stage 2a or 2b disease, without N-myc amplification, are also considered low risk and have excellent outcomes with surgical treatment. Those with amplification and favorable histology are still considered low risk, whereas those with N-myc amplification and unfavorable histology are high risk. Infants with stage 2 disease are considered low risk as well, regardless of N-myc amplification. Infants presenting with stage 4S disease have high rates of spontaneous regression, and high survival rates, as long as the tumor lacks N-myc amplification [9•].

N-myc nonamplified stage 3 disease constitutes the intermediate-risk group. Infants with stage 4s and unfavorable histology, or a diploidy index of 1, also are intermediate risk. Finally, infants with nonamplified stage 4 disease are intermediate risk. The intermediate-risk group patients have an excellent prognosis, with an 80 to 90% survival after a 9-month course of chemotherapy using cisplatin, etoposide, cyclophosphamide, and doxorubicin [9•]. Currently, there is a phase III COG trial attempting to reduce therapy in this favorable group using a four-cycle treatment in patients with favorable biology and eight cycles for those with diploid tumors or unfavorable histology.

The high-risk group is composed of infant patients with N-myc amplification and stage 3, 4, or 4s tumors, as well

Figure 1. CT of neuroblastoma arising from right adrenal gland

as older patients with N-myc amplification and stage 2, 3, or 4 tumors. Children with stage 4 disease have a poor prognosis with an overall survival of 20 to 35% [9•]. However, survival rates have improved with intense research efforts in the past several decades. Treatment of stage 4 patients generally consists of intensive induction chemotherapy, high-dose myeloablative therapy with allogeneic or autologous bone marrow or peripheral blood stem cell transplant, surgery, radiation therapy in some cases, and maintenance or biologic therapy to eradicate minimal residual disease. Dose intensity of induction chemotherapy has been shown to correlate with response and with event-free survival [10]. A combination of cyclophosphamide, vincristine, and doxorubicin alternating with cisplatin and etoposide showed good results in a pilot study and is currently undergoing phase III evaluation in a national COG study [9•]. Myeloablative consolidation has been supported by two randomized controlled trials. In a study by the European Neuroblastoma group, unpurged autologous bone marrow transplantation increased the mean progression-free survival time in advanced-stage patients in remission compared with patients who did not receive further therapy after chemotherapy [12]. A CCG study of myeloablative therapy and autologous bone marrow transplant showed greater event-free survival than chemotherapy alone [13].

Stage 4s neuroblastoma is usually a disease process with tumor regression and a favorable outcome. Despite this fact, some patients will have progression of their disease requiring cytotoxic therapy. Schleiermacher *et al.* [14••] retrospectively reviewed 94 patients from 1990 to 2000 with stage 4s neuroblastoma. Sixty percent of these patients presented with symptoms requiring therapy. Combinations of liver radiotherapy, cyclophosphamide–vincristine, and cyclophosphamide–etoposide were used. Overall survival most strongly correlated with necessity of cytotoxic therapy, 100% versus 80% for those not requiring treatment. For those requiring treatment, a more intensive cyclophosphamide–etoposide regimen appeared to result in better overall survival and less need for second-line therapy [14••].

For patients less than 1 year of age, unresectable neuroblastoma has a poorer prognosis than those that can be resected. Rubie *et al.* [15] examined 39 patients with nonresectable N-myc nonamplified tumors in children less than 1 year of age. They demonstrated that approximately half the patients were able to undergo resection after low-dose cyclophosphamide and vincristine. Infants were thus spared the toxicity of standard chemotherapy regimens with 100% overall survival [15].

To define the role of surgery in neuroblastoma, La Quaglia *et al.* [16••] published their 23-year experience on the effects of gross total tumor resection on survival in 141 high-risk patients. The probability of local progres-

sion of disease was 10% compared with 50% ($P < 0.01$) in 103 patients who had gross total resection versus those without total resection. The overall survival was 50% compared with 11% ($P < 0.01$) [16••].

The impact of radiotherapy on residual primary disease sites in patients with high-risk neuroblastoma treated on CCG protocol 3891 was examined by Haas-Kogan *et al.* [17••]. External beam radiotherapy was administered to all patients with gross residual disease after induction chemotherapy and surgery whether they were receiving standard chemotherapy or myeloablative therapy. The additional 10 Gy of radiation these patients received significantly reduced local disease recurrence [17••].

Paulino [18] reviewed 29 patients from a single institution using radiation therapy for palliation of symptomatic metastatic disease. A response rate of 79% and 77% was seen in bony and soft tissue sites, respectively. Central nervous system symptoms were also improved [18]. Despite limitations in this review, there is some suggestion that radiotherapy may play a role in palliative treatment of metastatic neuroblastoma.

Recurrent disease

Lau *et al.* [19] examined their series of patients with recurrent neuroblastoma and attempted to identify factors influencing survival time after relapse. Thirty-one patients were evaluated and 77% relapsed within the first 24 months, with 28 patients dying of progressive disease. Univariate analysis found N-myc amplification, chromosome 1p deletion, recurrence within 12 months of diagnosis, and recurrence within 6 months of stem cell transplant all to be significant factors in decreasing survival time after relapse. Salvage therapy was found to prolong survival in patients who were not N-myc amplified and in those who were greater than 6 months post stem cell transplant [19].

Kramer *et al.* [20] retrospectively analyzed patients taking oral topotecan for relapsed neuroblastoma resistant to conventional treatment. In 5 of 20 patients, modest radiographic improvements of tumor burden were demonstrated. Side effects of the drug included diarrhea and myelosuppression [20]. These findings suggest some benefit and warrant further investigation.

Secondary malignancy

Secondary malignant neoplasms in neuroblastoma patients who have received radiotherapy and chemotherapy were reviewed by Rubino *et al.* [21•] Five hundred forty-four patients with a primary neuroblastoma diagnosed between 1948 and 1986 were included. Twelve patients developed secondary neoplasms, including five thyroid and three breast cancers. Radiation therapy was found to be an important risk factor for developing secondary malignancy, whereas chemotherapy was

not [21•]. These findings reflect the importance of long-term surveillance of patients with neuroblastoma for secondary neoplasms.

Novel therapies

Survival for high-risk neuroblastoma remains less than 50%, and new approaches are needed. Retinoid therapy, new forms of drug delivery, and immunologic therapies are the newest weapons under investigation.

Research into the use of retinoids in the treatment of neuroblastoma has remained a major focus of current investigations [22••]. Since a phase III randomized trial has shown that high-dose, pulse therapy with 13-*cis*-retinoic acid given after intensive chemoradiotherapy significantly improved event-free survival in high-risk neuroblastoma, research has centered on elucidating the mechanism of action of the retinoids and on developing synthetic analogues of retinoic acid in the hope of decreasing side effects and improving efficacy. Microarray technology has been used to screen for genes that are important in neuroblastoma differentiation induced by 13-*cis*-retinoic acid. Using this screening device, a number of genes have been found to be either up- or down-regulated in this process [23•]. Other investigators have demonstrated that these genes interfere with cell growth by inducing neuronal differentiation in N-type neuroblastoma cells and apoptosis in S-type neuroblastoma cell lines [24]. This year marked the completion of a phase I trial of fenretinide, a synthetic retinoid that induces apoptosis that showed administration up to 4000 mg/m²/d over 28 days, followed by a 7-day interruption, resulted in manageable toxicity with plasma concentrations adequate to induce apoptosis in neuroblastoma cell lines [25•]. Continued research into the mechanism of action of fenretinide has demonstrated that it up-regulates the stress-induced transcription factor GADD153 and the Bcl-2-related protein Bak. Overexpression of these factors increases sensitivity to fenretinide-induced apoptosis. The targeting of GADD153 and Bak in neuroblastoma cells may provide novel pathways for the development of drugs inducing apoptosis of neuroblastoma with improved specificity [26].

Efforts to develop immunotherapy for neuroblastoma resulted in the development of a DNA vaccine that induces protection against metastatic neuroblastoma in a mouse model [27••]. These results were achieved with a tyrosine hydroxylase-based DNA vaccine enhanced with posttranscriptional regulatory-acting RNA elements combined with an antibody-cytokine fusion protein targeting interleukin-2. Vaccinated mice were protected from hepatic metastases. Both T-cell and natural killer cell-dependent mechanisms were involved in the induction of a systemic tumor-protective immunity. Similarly, anti-GD2 monoclonal antibody immunotherapy has been shown to dramatically reduce the metastatic spread

of neuroblastoma and prolong survival in a dose-dependent manner in a mouse model. However, neither macrophages nor NK cells appeared to contribute to the protective effect of antibody treatment *in vivo*, suggesting either an involvement of granulocytes or a complement-mediated cytotoxicity towards neuroblastoma cells.

Another novel form of therapy for neuroblastoma that has recently been investigated is the targeted delivery of antisense oligonucleotides. The primary factor limiting oligonucleotide application *in vivo* is their rapid degradation by cellular nucleases. To overcome this limitation, investigators have encapsulated the antisense oligonucleotides within liposomes, increasing their stability. Using this technique, C-myb antisense oligonucleotides have been encapsulated [28]. These liposomes were then externally coupled to a monoclonal antibody specific for the neuroectodermal antigen disialoganglioside GD2 resulting in creation of anti-GD2-targeted liposomes. These liposomes were characterized by high loading efficiency, small particle size, and good stability. *In vitro*, they were able to be selectively delivered to neuroblastoma cells and inhibit cell proliferation by down-modulation of c-myb protein expression. *In vivo* studies are ongoing.

New forms of drug delivery have been applied to etoposide, in an attempt to rationally design a prodrug with diminished toxicity and improved efficacy. Two novel approaches have been taken, including development of a hydrolytically activated prodrug by functionally blocking VP-16 by a carbonate linker [29••] and development of 3,4 dihydroxy-phenyl carbamate derivative of etoposide, which is activated by tyrosine hydroxylase, a neuroblastoma enzyme [30]. Both prodrugs have been demonstrated to be efficacious *in vitro*, whereas the hydrolytically activated prodrug has been shown to have improved efficacy and reduced side effects when compared with parenterally administered VP-16 in a mouse model. These feasibility studies warrant further investigation.

Conclusion

Neuroblastoma remains an enigmatic tumor. Continued investigation into the molecular mechanisms underlying the biology of this tumor has begun to shed light onto the mysteries of its variable natural history. With the advent of the use of powerful molecular tools such as gene arrays, continued progress in this field is assured. Translation of these findings into efficacious therapeutic modalities remains a slow and gradual process focused on risk stratification-based treatment. Continued work developing the novel therapies outlined in this update hold promise for continued progress in the treatment of neuroblastoma.

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