Pediatr Blood Cancer

MEETING REPORT
Neuroblastoma Progress on Many Fronts: The Neuroblastoma Research Symposium

Luke Wylie, BA, BS, [1,2] and Anna Philpott, BA, PhD[1]*

Neuroblastoma (NBL) is a pediatric tumor of infancy derived from precursor cells of the sympathetic nervous system. Clinicians and researchers in developmental biology and genetics recently met to facilitate meaningful crosstalk and to discuss considerable progress made in the clinical treatment and basic biology of NBL. For instance, discoveries in familial NBL have identified genetic aberrations in Phox2b and Alk that predispose to NBL, while advances in epigenetics and MYCN regulation have also offered insight into NBL pathogenesis and future treatment. Moreover, novel therapeutic avenues are also being explored, including targeted immunotherapies, and innovative radiotherapeutic and chemotherapeutic approaches. This multi-disciplinary meeting was convened to aid the transfer of new biological findings into the clinic and to use clinical advances to inform the basic biological understanding of this devastating disease. Pediatr Blood Cancer

Key words: molecular biology of neuroblastoma; neuroblastoma; neuroblastoma biology

INTRODUCTION

Neuroblastoma (NBL) is a tumor of the autonomic nervous system and is the most common cancer diagnosed in the first year of life [1]. Children with high risk NBL have a 5 year survival of only around 40% [2]. Mortality rates in these patients with high risk NBL have improved only modestly over the last 30 years, calling for continued and innovative research in the NBL field. On Friday, December 3rd 2010, researchers interested in unraveling the pathogenesis and improving future treatments for NBL patients gathered for a symposium in Cambridge, UK organized by The Neuroblastoma Society. The Neuroblastoma Research Symposium, will be a biennial meeting alternating with the International Advances in Neuroblastoma Research Symposium providing a forum to for basic scientists, clinicians, and charity leaders to discuss immediate and urgent topics in NBL.

Andrew Pearson (ICR, Sutton) began the meeting with a comprehensive overview of high risk NBL treatment, past, present, and future. Pearson praised SIOPEN (International Society of Paediatric Oncology—European Neuroblastoma) and its American counterpart Children's Oncology Group (COG), emphasizing how their collaborative efforts are vital for advancing care for all children. Pearson also lauded the efforts of the NBL community in creating the International Neuroblastoma Risk Group (INRG) classification system, which stages patients before treatment begins based on non-invasive imaging and clinical factors allowing for more consistent stratification and inclusion of patients where surgery is not needed, but are simply observed [2,3]. However, he called for improved treatment standards for patients with tumors containing MYCN amplification and activating anaplastic lymphoma kinase (Alk) mutations, which may have treatment susceptibilities targeted to their unique pathogenesis.

NEUROBLASTOMA, A CANCER OF DEVELOPMENT

NBL is derived from precursor cells of the sympathetic nervous system which require a network of transcription factors to properly differentiate into mature sympathetic neurons. Hermann Rohrer (MPI for Brain Research, Frankfurt) described the role of one of these transcription factors, PHOX2B, which has also been associated with familial forms of NBL [4] and previously shown to regulate proliferation and differentiation of neuronal precursors [5–7]. By elegant work in chick sympathetic neuroblasts, Rohrer showed that overexpression of wild-type Phox2b caused reduced proliferation, but recapitulation of PHOX2B mutations found in familial NBL caused increased proliferation [8]. Rohrer also showed that inhibition of Alk, which has recently been shown to be hyperactive in both familial and sporadic forms of NBL [9], results in decreased proliferation of sympathetic neurons, but the role of ALK within development has yet to be determined.

The sympathetic neuroblasts characterized by Rohrer are derived from neural crest cells, which are a population of multipotent migratory stem cells. Ben Simons (University of Cambridge) gave a broad overview of mechanisms of stem cell maintenance. Lineage labeling and long-term follow up has allowed Simons and colleagues [10] to demonstrate in several tissues that resident stem cell populations are maintained by undergoing balanced stochastic fate choices, rather than each individual cell being programmed to divide asymmetrically, suggesting that at least some populations of stem cells are more dynamic and heterogeneous than previously thought. David Kaplan (SickKids, Toronto) identified commonalities between neural crest cells and tumor-initiating cells (TICs) found in the bone marrow from metastatic NBL patients. Kaplan showed that TICs not only retain neural crest markers, but also express B-cell markers [11]. Although the existence of NBL TICs with a B-cell phenotype is still quite controversial, this could potentially open up new avenues for treatment in patients with disseminated disease. Indeed, B-cell specific drugs used for treatment in

[1]Department of Oncology, University of Cambridge, Hutchison/Medical Research Council (MRC) Research Centre, Cambridge, UK; [2]National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Grant sponsor: National Institutes of Health Oxford-Cambridge Scholars Program; Grant sponsor: Medical Research Council; Grant number: G0700758.

*Correspondence to: Anna Philpott, Department of Oncology, University of Cambridge, Hutchison/Medical Research Council (MRC) Research Centre, Cambridge CB2 0XZ, UK.
E-mail: ap113@cam.ac.uk

Received 22 June 2011; Accepted 4 August 2011

DOI 10.1002/pbc.23329
Published online in Wiley Online Library (wileyonlinelibrary.com).

lymphoma, such as Rituximab and Milatuzumab, have also shown to be active in NBL TICs and may provide additional benefit to patients with metastatic NBL.

EPIGENETIC DYSREGULATION IN NEUROBLASTOMA

Epigenetic regulation of gene expression is poorly understood, yet its control is vital for normal cell function, and is often awry in various cancers. The polycomb repressor complex 2 (PRC2) is a complex of proteins that remodels chromatin and silences expression of various genes including putative tumor suppressors. Carol Thiele (NCI, Bethesda) presented work on one of these putative tumor suppressors, CASZ1, found within the 1p36 region, that is, commonly lost in high risk NBL. While one allele of CASZ1 is inactive from 1p36 loss, the other allele is often genetically intact, suggesting an alternative means of repression. The PCR2 complex protein Ezh2 is responsible for chromatin silencing by histone methylation and can be inhibited with the drug DZNep, a global inhibitor of histone methylation [12]. DZNep administration to NBL cell lines caused enhanced CASZ1 expression and NBL differentiation, suggesting that Ezh2 may be repressing the second allele of CASZ1.

Continuing on the theme, Arturo Sala (UCL, London) presented a similar model of epigenetic repression. In NBL, MYCN overexpression was found to be associated with lower expression of a putative tumor suppressor Clusterin. Sala's group found that N-Myc directly binds to the Clusterin promoter region. They found that N-Myc-mediated repression of Clusterin could be abrogated by shRNA against Suz12, a repressive component of the PCR2 complex [13]. Further, administration of HDAC inhibitors, which inhibit PRC2 activity, caused increased expression of Clusterin and subsequent inhibition of tumor growth. Importantly the inhibition of tumor growth seen with HDAC inhibitors was reversed with shRNA against Clusterin.

Both the work of Thiele and Sala highlight epigenetic regulation as a potential therapeutic avenue for NBL. Individual patient samples could be screened for expression of tumor suppressors such as Casz1 or Clusterin after HDAC inhibition. This might indicate therapeutic responsiveness, providing a more personalized treatment approach for NBL patients.

MYCN

MYCN is part of a family of Myc proteins including c-Myc, and amplification is strongly correlated with poor prognosis in NBL. Gerard Evan's group (Dept of Biochemistry, University of Cambridge) created a mouse line with inducible repression of c-Myc. When c-Myc was repressed, essentially all cell proliferation was halted including in the epidermis and gut epithelium. Remarkably, the animals remained in good health and the effects were completely reversible. In mouse lung cancer models very impressive tumor regression was observed during c-Myc repression [14]. Evan's group also observed that when Myc inhibition was restricted to tumor cells, the surrounding tumor microenvironment collapsed, suggesting that c-Myc in tumor cells may be necessary to maintain the tumor microenvironment. While Myc inhibition appears to be an attractive treatment in various cancers there are some caveats. Transcription factors like Myc are currently inaccessible as drug targets and Myc inhibition may not be viable for treatment of pediatric cancers due to its vital role in normal development.

How MYCN regulates tumorigenesis in NBL was also discussed. Frank Speleman (Centre for Medical Genetics, Ghent) analyzed miRNA profiles in NBL that correlated with MYCN status and poor prognosis. One strongly correlated group were miRNAs from the 17 to 92 cluster, which coordinate control of proliferation, apoptosis, and angiogenesis in NBL cells [15]. Speleman also compared miRNA expression patterns between fetal neuroblasts and NBL. They found that miRNA 204 was differentially expressed and could downregulate Phox2b, which aligned well with Rohrer's data. To evaluate MYCN overexpression within various pediatric cancers Louis Chesler (ICR, Sutton) is creating a series of Genetically Engineered Mouse Models (GEMMs). Currently the only successful mouse model of NBL results from the MYCN gene regulated by the tyrosine hydroxylase promoter, predominantly expressed within neural crest cells [16]. Chesler is tying MCYN expression to promoters of genes involved in early neural crest. This may yield further insight into whether neural crest cells show a critical period of susceptibility to tumorigenesis.

FUTURE THERAPEUTICS

A strong focus on the basic science behind NBL was complemented by extensive discussion of new treatments for NBL. Immunotherapy has become a viable option for NBL, as patients given chimeric human-mouse monoclonal antibodies against GD2, a surface glycolipid on NBL cells, have shown improved outcomes [17]. NBL is a strongly immunogenic tumor that often has significant lymphocyte infiltration on biopsy at diagnosis or following chemotherapy. Juliet Gray (University of Southampton) is attempting to leverage this fact by utilizing the patient's own immune system [18]. By administering immune costimulatory molecules to amplify the endogenous immune response, the Gray lab has seen some promising long-term results in mouse NBL models. In a related approach, John Anderson (UCL, London) is attempting to create designer T-cells for use against tumor-specific antigens such as GD2 in NBL. By creating a fusion protein of the variable region of an antibody against an antigen and the intracellular zeta-chain domain of CD3, modified T-cells will mount an immune response against NBL specific antigens.

In addition, there were new advances made in more standard treatments. Metaiodobenzylguanidine (mIBG) is a radiolabeled molecule that is similar to norepinephrine and is taken up by NBL cells. Using more potent radioactive forms of mIBG can cause DNA damage. Rob Mairs (Beatson Laboratories, Glasgow) presented initial data that by combining mIBG with drugs that inhibit DNA repair, such as PARP and topoisomerase inhibitors, synergistic effects were achieved in NBL. Emma Bell and colleagues (NICR, Newcastle) found that retinoic acid, part of the treatment for minimal residual disease in NBL, activated the PPAR-delta pathway. PPAR-delta signaling has numerous cellular functions including proliferation and metabolism. Therefore, inhibiting PPAR-delta signaling may be a viable adjunct therapy for minimal residual disease after treatment with retinoic acid.

CONCLUSIONS

The diverse presentation and variable natural history of NBL make this disease a particular challenge to scientists and

clinicians alike. However, it is clear from this meeting that a deeper understanding of both the underlying normal development of the sympathetic nervous system and changes in biology of NBL cells at different stages of this disease will aid considerably in our understanding and treatment of NBL in the future. The Neuroblastoma Society (http://www.nsoc.co.uk) plans to organize the Neuroblastoma Research Symposium every 2 years. The next meeting will be in Autumn 2011 and will focus on immunotherapy, gene expression profiling, and emerging treatments for neuroblastoma.

ACKNOWLEDGMENT

The authors would like to thank both the participants of the Neuroblastoma Symposium and Deborah Tweddle and Guy Blanchard for critical reading of this manuscript. Luke Wylie is supported by the National Institutes of Health Oxford-Cambridge Scholars Program. Anna Philpott is supported by MRC research grant G0700758.

References

1. Maris JM. Recent advances in neuroblastoma. N Engl J Med 2010;362:2202–2211.
2. Monclair T, Brodeur GM, Ambros PF, et al. The International Neuroblastoma Risk Group (INRG) staging system: An INRG Task Force report. J Clin Oncol 2009;27:298–303.
3. Cohn SL, Pearson AD, London WB, et al. The International Neuroblastoma Risk Group (INRG) classification system: An INRG Task Force report. J Clin Oncol 2009;27:289–297.
4. Mosse YP, Laudenslager M, Khazi D, et al. Germline PHOX2B mutation in hereditary neuroblastoma. Am J Hum Genet 2004;75:727–730.
5. Dubreuil V, Hirsch MR, Pattyn A, et al. The Phox2b transcription factor coordinately regulates neuronal cell cycle exit and identity. Development 2000;127:5191–5201.
6. Dubreuil V, Hirsch MR, Jouve C, et al. The role of Phox2b in synchronizing pan-neuronal and type-specific aspects of neurogenesis. Development 2002;129:5241–5253.
7. Trochet D, Hong SJ, Lim JK, et al. Molecular consequences of PHOX2B missense, frameshift and alanine expansion mutations leading to autonomic dysfunction. Hum Mol Genet 2005;14:3697–3708.
8. Reiff T, Tsarovina K, Majdazari A, et al. Neuroblastoma phox2b variants stimulate proliferation and dedifferentiation of immature sympathetic neurons. J Neurosci 2010;30:905–915.
9. Mosse YP, Laudenslager M, Longo L, et al. Identification of ALK as a major familial neuroblastoma predisposition gene. Nature 2008;455:930–935.
10. Lopez-Garcia C, Klein AM, Simons BD, et al. Intestinal stem cell replacement follows a pattern of neutral drift. Science 2010;330:822–825.
11. Hansford LM, McKee AE, Zhang L, et al. Neuroblastoma cells isolated from bone marrow metastases contain a naturally enriched tumor-initiating cell. Cancer Res 2007;67:11234–11243.
12. Miranda TB, Cortez CC, Yoo CB, et al. DZNep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation. Mol Cancer Ther 2009;8:1579–1588.
13. Chayka O, Corvetta D, Dews M, et al. Clusterin, a haploinsufficient tumor suppressor gene in neuroblastomas. J Natl Cancer Inst 2009;101: 663–677.
14. Soucek L, Whitfield J, Martins CP, et al. Modelling Myc inhibition as a cancer therapy. Nature 2008;455:679–683.
15. Mestdagh P, Fredlund E, Pattyn F, et al. MYCN/c-MYC-induced microRNAs repress coding gene networks associated with poor outcome in MYCN/c-MYC-activated tumors. Oncogene 2010;29:1394–1404.
16. Weiss WA, Aldape K, Mohapatra G, et al. Targeted expression of MYCN causes neuroblastoma in transgenic mice. EMBO J 1997;16:2985–2995.
17. Yu AL, Gilman AL, Ozkaynak MF, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. N Engl J Med 2010;363:1324–1334.
18. Gray JC, Kohler JA. Immunotherapy for neuroblastoma: Turning promise into reality. Pediatr Blood Cancer 2009;53:931–940.