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Meeting Report - 3rd Neuroblastoma Research Symposium, Liverpool, 6-7th November, 2013

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Neuroblastoma accounts for around 15% of childhood cancer deaths. Survival rates are now slowly improving, but remain dire with less than 50% overall-survival for high-risk neuroblastomas [1]. Scientists and clinicians gathered at the 3rd Neuroblastoma Research Symposium in Liverpool on 6th-7th November 2013, organised by The Neuroblastoma Society charity, to discuss and share advances in the understanding of neuroblastoma biology and in the development of potential new therapies.

Epigenetic landscape of neuroblastoma

Driver genetic mutations in neuroblastoma are rare [2], while gene silencing by methylation is emerging as an important characteristic of high-risk neuroblastoma development. Jo Vandesompele (Ghent University) explained that aberrant DNA methylation patterns may contribute to the neuroblastoma phenotype by down-regulating specific genes and microRNAs involved in neuroblastoma biology. Most importantly, DNA methylation may be used as biomarkers to refine neuroblastoma risk classification, although the independent prognostic value of such methylation signatures over already known established risk factors remains to be established [3]. Further supporting the notion that epigenetic mechanisms contribute to neuroblastoma pathogenesis, Gudrun Schleiermacher (Institute Curie) reported that new mutations in tumours at relapse are rare, while histone methyltransferase (HMT) activity was often elevated. Histone methylation is also responsible for tumour suppressor gene silencing as highlighted by Karim Malik (University of Bristol), who showed an example of a gene encoding a pro-apoptotic protein repressed by HMTs. Drugs inhibiting HMTs were able to reactivate gene expression and trigger apoptosis, emphasizing the potential of epigenetic modulation for neuroblastoma therapies. It is hoped that future work in the epigenetics field will identify potential new drug targets. One possibility is EZH2, a component of the polycomb repressor complex 2 that interacts directly with MYCN to mediate transcriptional repression. A functional characterisation of genes differentially methylated between neuroblastoma prognostic sub-groups as well as between *MYCN*-amplified and *MYCN*-non amplified cases will provide further candidates.

The role of MYCN in childhood cancers

Historically, inhibiting MYC proteins with small molecules has proved difficult due to the complex nature of targeting transcription factor protein-protein and protein–DNA interactions. Evon Poon (Institute of Cancer Research) presented an elegant study describing how *MYCN*-amplified neuroblastoma cells depend on Aurora kinase A (AURKA) to protect MYCN from FBW7 mediated degradation. AURKA inhibitors promote conformational changes within

the kinase domain of AURKA, blocking MYCN binding and destabilising the oncoprotein [4]. Jo Vandesompele introduced another promising avenue, presenting newly identified miRNAs that interact with MYCN transcripts.

Disruption of MYCN activity in *MYCN*-amplified neuroblastoma cells induces growth arrest, differentiation and apoptosis. Marie Arsenian Henriksson's group (Karolinska Institute) have shown that MYCN knockdown or inhibiting the MYCN-MAX interaction using the inhibitor 10058-F4 induces the formation of lipid vesicles in the cytoplasm of neuroblastoma cells. Proteomic analysis revealed that reduction of MYCN leads to a decrease in respiratory chain and β-oxidation of fatty acids associated proteins [5]. Targeting MYCN activity with small molecule inhibitors requires a comprehensive model of both the mechanisms governing MYCN protein stability and the pathways influencing MYCN inhibition.

MYCC and MYCN are oncogenic drivers in medulloblastoma, a childhood tumour of the cerebellum. Steve Clifford (Newcastle University) explained that while *MYCC* amplification is strongly associated with poor prognosis, the significance of *MYCN* amplification for prognosis is dependent on additional factors, such as metastatic status [6]. Over-expression of MYC has paradoxical effects on cancer cells: driving proliferation and sensitising cells to apoptosis. Current studies targeting MYCN do not address how to inhibit MYCN driven proliferation without protecting cells from apoptosis. Future studies must precisely define the relevant pathways downstream of MYCN and aim for a comprehensive understanding of how developmental differences and additional risk factors influence the aggressiveness of MYCN driven malignancies.

Alongside MYCN, novel targets presented at the meeting included TAK1, Wee1 and PI3 kinase (Daniel Morgenstern, GOSH), BRD4 (Emma Bell, DKFZ) and p53-MDM2 antagonists as inhibitors of MDR-1 (Lindi Chen, Newcastle University). Taken together, these presentations had a clear message: Future successful treatment of neuroblastoma depends on intelligent design of drug combinations.

In-vivo neuroblastoma models

Getting a new drug into clinical trial requires proof-of-response in preclinical animal models. Laura Danielson (Institute of Cancer Research) provided an overview of transgenic neuroblastoma murine models. The TH-*MYCN* mouse was the first and is now well-established [7]. With advances in technology and the identification of further genetic/biological aberrations in neuroblastoma, additional transgenics are emerging (e.g. ALK[F1147L], LIN28B, Caspase-8 deficient, MYCN/NCYM) [8-12]. The utility of the ALK model is of particular interest as ALK is a novel therapeutic target in neuroblastoma. Suzanne

Turner (University of Cambridge) presented an overview of the well-established ALK induced mechanisms in Anaplastic Large Cell Lymphoma (ALCL) and an encouraging example of how the NPM-ALK transgenic mouse enabled the successful treatment of a relapsed ALCL patient with Imatinib [13].

A major limitation of neuroblastoma murine models has been the lack of distant metastases; however both the caspase-8 deficient and MYCN/NCYM models appear to have overcome this and may prove highly useful in the future. Louis Chesler's group (Institute of Cancer Research) are establishing a doxycycline-regulatable MYCN-off model that will enable greater control and understanding of MYCN during neuroblastoma tumourigenesis and oncogenic addiction.

Anti-GD2 immunotherapy is now routinely used in the clinic. Poster presentations at the symposium highlighted the ongoing efforts to enhance the efficacy of GD2 therapy by the co-administration of immune adjuvants. Juliet Gray (University of Southampton) discussed the importance of immunocompetent murine models, in view of the difficulties of recapitulating the immune microenvironment in vitro. The TH-*MYCN* mouse model was found to be the most comparable with neuroblastoma patients with respect to GD2 expression and the immune environment. More specifically, TH-*MYCN*$^{+/-}$ mice are preferred over TH-*MYCN*$^{+/+}$ mice due to longer tumour latency, enabling the development of a more mature immune system. The narrow therapeutic window in which to assess immunotherapy strategies due to tumour burden has been a major challenge. To overcome this, Gray and colleagues use a sub-therapeutic dose of cyclophosphamide (40mg/kg) to control tumour burden. The utility of the new generation of transgenic mouse models for studying immunotherapy remains to be established.

Violaine Sée (University of Liverpool) described the development of a metastatic model system in the chick embryo in which the behaviour and transport of fluorescently tagged neuroblastoma cells within the vascular system could be visualised. Cells with prior exposure to hypoxia were more likely to attach to vessel walls and invade local tissue, suggesting that hypoxia-activated genes could be therapeutic targets. Metastasis was also the focus of posters by Sue Burchill's group (University of Leeds), who are isolating stem cell-like neuroblastoma cells that have disseminated to the bone marrow, and by Rob Falconer's team (University of Bradford), who are developing inhibitors of the enzyme responsible for the production of cell-surface polysialic acid to reduce tumour cell dissemination [14].

Facilitated by Louis Chesler, lively discussions focused on the need for models not involving MYCN, representing the different subgroups or stages of disease (pre-treatment, minimal residual disease, relapse), and whether a "murine models consortium" should be established to help facilitate the development of such valuable research models. Further discussions touched on other cheaper models, primarily zebrafish, *Xenopus* frog and fruit fly, each of which offer something unique to cancer modelling, particularly for understanding conserved pathways affected in neuroblastoma. However novel routes to treatment would continue to require *in vivo* mouse models.

Differentiating neuroblastoma

The administration of 13-cis-retinoic acid (RA) induces neuroblastoma differentiation and leads to improved survival rates [15]. Gareth Veal (Newcastle University) demonstrated the importance of monitoring the ratio of parent drug to metabolite obtained in plasma and of subsequent adjustments to the second dosage of RA to achieve the specified therapeutic dose [16]. The need to consider carefully drug formulation when administered to children was discussed. Andy Stoker (UCL) presented mechanisms for how oxovanadium compounds, protein tyrosine phosphatase inhibitors given to patients with diabetes, drive neuroblastoma differentiation and senescence in combination with RA [17].

Approaching neuroblastoma therapy with expertise from developmental biology, Marthe Howard (University of Toledo) and Anna Philpott (University of Cambridge) focused on Hand2 and ASCL-1, respectively. Both transcription factors are required for the differentiation of sympathetic neurons [18] and are implicated in aggressive neuroblastomas. The talks reported progress towards a mechanistic understanding of how neuronal differentiation occurs normally and hence how this process could be encouraged therapeutically in neuroblastoma. Howard showed that the key neuroblastoma genes MYCN, ALK and Phox2B are direct targets of Hand2, and that siRNA knockdown of Hand2 in cell lines led to a reduction in protein levels of all three. Hence Hand2 emerges with therapeutic potential.

Anna Philpott reported that both the amount of ASCL-1 and its phosphorylation by CDK2, control the fate of sympathetic neurons. Dephosphorylation of ASCL-1 leads to cell cycle exit and differentiation, both in normal development and in neuroblastoma cells, suggesting a mechanism for why CDK inhibitors are effective in neuroblastoma [19]. Through mechanisms that are not yet fully understood, RA works synergistically with CDK inhibition and potentially ASCL-1 dephosphorylation, with Hand2 knockdown and with oxovanadium

compounds above. Understanding this synergy could bring significant improvements to differentiation therapies that are urgently needed to reduce the risk of relapse.

Conclusion

Finding therapeutic synergies through a mechanistic understanding of how drug combinations affect neuroblastomas, and developing and testing these in a range of animal models, is the most promising route to improve current therapies. Therapeutic synergies will bring efficacy but also specificity to tumour cells, crucial for reducing long-term side effects in pediatric patients. A mechanistic understanding is essential for identifying the likely key pharmacokinetic measures that will allow precise monitoring of drug efficacy and patient differences in response. The 4th Neuroblastoma Research Symposium will be held in the autumn of 2015 in Newcastle, UK.

Acknowledgements

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