Translating childhood brain tumour research into clinical practice: the experience of molecular classification and diagnostics

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Abstract

Diagnosis and treatment of paediatric brain tumours has shown limited progress over the last half century. However, in the past 10 years the development of molecular techniques for investigating these tumours has expanded exponentially. The use of methylation profiling, gene expression analysis and the identification of gene fusions are forming the basis for improved diagnostic criteria and new treatment approaches. Knowledge and practice in this area is now beginning to expand beyond the research field and into the clinical setting. As the Chief Medical Officer highlighted in July 2017, an understanding of molecular medicine and its implications for both patients and the health economy is important for all clinicians. In this article, we summarise important recent advances in molecular medicine in childhood brain tumour research using the three most common types of paediatric brain tumour; pilocytic astrocytoma, medulloblastoma and ependymoma as illustrative examples.

Keywords Brain tumours; DNA methylation; ependymoma; fusion genes; medulloblastoma; molecular diagnostics; pilocytic astrocytoma

Introduction

On the 4th July 2017, Dame Sally Davies, the Chief Medical Officer for NHS England set out a five-year plan to enable whole genome sequencing for every cancer patient. In her report 'Generation Genome', she states that *"Genomics is not tomorrow. It's here today"*. This applies not only to genome sequencing but to molecular diagnostics in medicine as a whole. This article outlines how approaches to molecular medicine have developed over the last decade, applied to the field of paediatric brain tumours.

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Tumours of the brain and spinal cord are the second most common type of cancer in childhood. Of the 1800 new childhood cancer diagnoses reported each year, brain tumours account for 26%. Whilst survival rates for the most common type of cancer, leukaemias, have been improving, with 10-year survival increasing from 27% in 1971 to 81% in 2005, the improvement for brain tumours has been more modest, increasing from 38% in 1971 to 68% in 2005. The mainstays of therapy for childhood brain tumours include surgery, chemotherapy and radiotherapy, and this has remained largely unchanged for the last 50 years. All of these approaches have significant side effects on the developing brain and, as a consequence, brain tumours in childhood are associated with significant morbidity in addition to mortality. There is an urgent need for the development of new, effective interventions to both minimize the collateral damage to the surrounding brain and improve clinical outcomes, including long-term survival.

The relatively poor improvement in overall survival for children with brain tumours has led to an increased drive for research into these devastating diseases. In particular, the development of molecular classifications for several brain tumour types has provided increasing levels of detail about clinical and biological behaviour; removed some entities from the lexicon; and uncovered previously unrecognized subtypes. At present the development of effective new treatments from this wealth of molecular information has been modest, but it is hoped that these new approaches to classification will lead to better outcomes in the future. Recent developments in ependymoma, medulloblastoma and pilocytic astrocytoma, the three most common paediatric brain tumours, provide excellent illustrative examples of the progress of molecular profiling of paediatric brain tumours and form the focus of this review. This progress will be illustrated through the description of the role of two important genetic processes; DNA methylation and the formation of fusion genes.

DNA methylation profiling

Methylation is an epigenetic modification. It is a process whereby DNA can be modified without changing the underlying base sequence, by adding a chemical 'tag' to part of the sequence. Epigenetic modification leads to phenotypic variation, the process by which different cells of an organism, all with identical genetic information, are radically different from each other.

The addition of a methyl group or several methyl groups to a DNA molecule can alter gene expression and function. Alterations to DNA methylation patterns have previously been described in the developing fetus and also in ageing. It is now believed to play a crucial role in cancer and has become a particular focus for cancer research in both adults and children. Indeed, with only around 20,000 human genes it is likely that epigenetic modifications play a very important role in creating diversity in their expression.

Methylation patterns vary and this variation can be determined by the use of DNA methylation arrays. An array consists of a synthetic chip covered in microscopic probes specific for predefined DNA sequences. One probe contains the methylated sequence and a partner probe contains the unmethylated sequence. DNA is hybridized to its complementary probe before

Please cite this article in press as: Ritzmann TA, Grundy RG, Translating childhood brain tumour research into clinical practice: the experience of molecular classification and diagnostics, Paediatrics and Child Health (2018), https://doi.org/10.1016/j.paed.2018.01.006

being stained with fluorescent dyes. The array is then imaged, so that the level of DNA methylation for each probe can be calculated according to the intensity of the dye. Currently, across one methylation array, 850,000 different probes can be interrogated to provide a wealth of information about the methylation characteristics of a tumour.

International research collaborations have resulted in the analysis of thousands of paediatric brain tumours using DNA methylation arrays. Sophisticated machine learning techniques have been developed to define characteristic methylation patterns for different paediatric brain tumours which can be used as a basis for their molecular classification. The use of this technique will be described in subsequent sections for both ependymoma and medulloblastoma.

Fusion genes

Fusion genes arise as a result of chromosomal rearrangement. One extreme way in which these rearrangements can occur is through a process called chromothripsis in which a chromosome is shattered following a severe insult to a cell. The shattered chromosome is then rearranged incorrectly, possibly through aberrant DNA repair pathways or replication mechanisms, resulting in genetic translocations, deletions or inversions. Fusion genes therefore represent the joining together of two previously separate genes. They can then develop a new or exaggerated function which may result in the development or maintenance of malignancy. Arguably the most famous fusion gene, first described in the 1980s, is the BCR-ABL1 fusion found on the Philadelphia chromosome in patients with Chronic Myeloid Leukaemia. Since then the discovery of new fusion genes in cancer has developed exponentially and as of 2017, the Mitelman database, a key archive cataloguing all of the documented cancer fusion genes, stated that 10,861 gene fusions had been described across many different tumour types.

Fusion genes can be identified using numerous techniques, including Fluorescent In-Situ Hybridisation (FISH), the Polymerase Chain Reaction (PCR) or RNA sequencing (RNA-Seq).

Some studies have suggested that tumours with certain fusion genes display specific methylation patterns as will be described in the following case studies.

Case studies of three brain tumour types

Ependymoma – molecular characterisation unveils multiple, location dependent subgroups with different clinical outcomes and identifies tumour driving gene fusions

Ependymoma is the second most common malignant brain tumour of childhood and is slightly more common in boys than in girls. Outcomes are poor, with a ten-year overall survival rate of around 60%. Relapses are common with a 50% risk of relapse and 25% five-year overall survival following relapse. Prognosis has previously been based on age, with younger children having poorer outcomes; tumour location, with spinal rather than intracranial tumours having the best outcomes; and the extent of surgical resection at initial surgery, with a complete removal of the tumour being associated with the best prognosis. Treatment can vary dependent on age. All children receive surgery, but those under 3 years receive chemotherapy, with a view to delaying or averting the need for radiotherapy. Children over three often receive radiotherapy. Despite treatments targeted at different age groups and focus on achieving complete surgical resection, outcomes still remain very poor, particularly following relapse.

Methylation profiling of extensive, international cohorts of ependymoma has suggested the existence of a number of ependymoma molecular subgroups. Each subgroup has specific associations with intracranial or spinal location and age, and demonstrate disparate clinical courses and outcomes (Figure 1). Four of the subgroups in particular, PF-EPN-A, PF-EPN-B, ST-EPN-RELA and ST-EPN-YAP, occur more frequently in children and young people and will be described in more detail.

PF-EPN-A and PF-EPN-B both arise in the posterior fossa of the cranial cavity and make up the vast majority of all posterior fossa ependymomas. PF-EPN-A are more aggressive tumours and disproportionately affect younger children. These tumours are invasive, occur laterally and can be difficult to resect. Treatment strategies focused on these tumours are often unsuccessful, resulting in rapid disease recurrence and progression. PF-EPN-B are commoner in older children and adults and appear to have much better outcomes. These tumours are less invasive and some researchers and clinicians have suggested that the only treatment required may be surgery. This viewpoint has not reached an international consensus, and will hopefully be addressed in new clinical trials.

ST-EPN-RELA and ST-EPN-YAP are tumours of the supratentorial region of the cranial cavity. Both of these tumour types are associated with fusion genes; ST-EPN-RELA with the *C11orf95-RelA* fusion and ST-EPN-YAP with various fusions of the gene *YAP1*. It has been postulated that these fusions are the oncogenic driving events for these ependymoma subtypes. So distinct is the ST-EPN-RELA group, that the World Health Organisation's classification of tumours of the central nervous system has recently been updated to include RELA fusion positive ependymomas as a separate disease entity. ST-EPN-RELA tumours appear to have very poor outcomes and present most commonly in younger children. ST-EPN-YAP tumours also present in young children, but are much less common and appear to be associated with better outcomes. However, because of the relative rarity of the YAP fused tumours the clinical outcomes for this subtype are still being fully discerned.

The discovery of these methylation subgroups and associated fusion genes provides some explanation as to why patients with ependymoma show such a heterogeneous clinical course. It is clear, with the lens of molecular profiling, that even within the broad category of ependymoma there are subgroups of disease, with distinct patterns of clinical behaviour; arising in different age groups; and exhibiting diverse clinical outcomes. This understanding is important as future research into effective treatments needs to take into account these subtypes to form valid conclusions. From a clinical perspective, it is important to be aware that the diagnosis of ependymoma itself does not necessarily indicate a particular prognosis, and that more detailed molecular analysis may provide a clearer guide for families and oncologists of likely outcomes. There is a move towards treatment strategies being guided by molecular subgroup in the case of PF-EPN-B and a potential reduction in therapeutic burden, but this is far from a consensus and is not part of recommended clinical practice in the UK. Unfortunately, novel or repurposed

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