The Genesis of Malignant Rhabdoid Tumours

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Malignant rhabdoid tumours (MRTs) are rare tumours found mainly in the kidneys and central nervous system, most commonly present in young infants and children and are typically associated with a very poor prognosis. Almost all rhabdoid tumours harbour a biallelic mutation of SMARCB1, a core subunit of the SWI/SNF chromatin remodelling complex. It has become apparent that epigenetic influences are a major driver of rhabdoid tumour growth. Current treatment regimens consist of aggressive multimodal therapies which can result in high treatment toxicity to patients. Only recently have specifically-designed treatment strategies been laid out for rhabdoid tumours in an official framework, and prognosis has consequently improved significantly. Most of these trials use molecular inhibitors to block tumour growth. Rhabdoid tumours and their oncogenic processes that drive their growth are the key targets for novel therapies and future treatment potential for patietns with MRTs.

Introduction

Malignant rhabdoid tumours (MRTs) are very rare but aggressive childhood cancers. MRTs are distinct from rhabdomyosarcomas (a cancer most commonly associated with rhabdoid cells). The term rhabdoid originally derived from cells that were 'rhabdoid' in nature when histological analysis of specimens was conducted by light microscopy. Rhabdoid cells are characterised by prominent nuclei, a single large nucleolus and a cytoplasm with globular eosinophilic inclusions (Barresi *et al.*, 2015).

MRTs are most commonly found in the kidneys (rhabdoid tumour of the kidney, RTK) and the central nervous system, in particular in the brain (atypical teratoid rhabdoid tumour, AT/RT). Rhabdoid tumours can also, more rarely, occur in extrarenal and extracranial soft tissues (Sredni and Tomita, 2015, Kerl *et al*., 2013). The presence of these rhabdoid cells and crucially the reduced expression of the SWI/SNF-related matrix-associated actin-dependent regulator of chromatin B1 (SMARCB1) tumour suppressor gene are the key diagnostic factors of MRTs (Kieran *et al*., 2012). Analyses of factors such as expression of smooth muscle actin and epithelial membrane antigen is also often used to differentially diagnose the tumours in histopathology (Margol and Judkins, 2014).

RTK was the first rhabdoid tumour to be discovered and was initially thought to be a variant of Wilms' tumour, which is itself a renal tumour (Beckwith and Palmer, 1978), but was soon found to be morphologically and biologically distinct (Weeks *et al*., 1989). AT/RT, on the other hand, had originally often been thought to be primitive neuroectodermal tumour until it was ultimately identified as a separate entity (Rorke *et al*., 1996). Although there has been debate on the distinctiveness of AT/RT and RTK (Parham *et al*., 1994), these two major forms have striking similarities and are seen as counterparts, and MRTs from all anatomic sites are considered to be the same tumour type (Grupenmacher *et al*., 2013).

The majority of rhabdoid tumours share a biallelic inactivation of the SMARCB1 gene (Versteege *et al*., 1998, Jackson *et al*., 2009). These genes encode proteins that are subunits of a larger chromatin remodelling complex (Roberts and Biegel, 2009). The exact mechanisms by which SMARCB1 deficiency causes tumour growth is not fully understood, but it is known that the cell cycle and sonic hedgehog pathways are affected (Mora-Blanco *et al*., 2014, Tsikitis *et al*., 2005). Ongoing research is being done to investigate intermediaries involved in these pathways with the aim of developing and testing the efficacy of drugs and novel therapeutic strategies to combat these devastating tumours. Some of these drugs include small molecule inhibitors such as EZH2 inhibitors (Knutson *et al*., 2014).

MRT usually presents at 3 years or younger (Ahmed *et al*., 2007, Woehrer *et al*., 2010). Prognosis is poor compared with other early paediatric cancers and survival rates are low. Currently, the European Rhabdoid Registry recommends multimodal treatment consisting of surgery, radiotherapy and chemotherapy (Frühwald and Krefeld, 2010), which is aggressive and can result in toxic deaths (Chi *et al*., 2009).

A review of the Irish cohort of MRT documented a total of 25 patients that were diagnosed with rhabdoid tumours from 1986-2013 in the Republic of Ireland, all under the age of 17 (Uwineza *et al*., 2014). According to Uwineza *et al.* (2014) there was an equal sex incidence with a mean age at diagnosis of 38.8 months. These patients were treated based on the anatomic site of the tumours, because there was no standardised treatment for rhabdoid tumours in place (such as the European Rhabdoid Registry, which was only founded in 2010). Ten patients (43.5%) relapsed and the mean time to death was just less than 9 months (Uwineza *et al*., 2014).

SMARCB1/SMARCA4 as Tumour Suppressors

Chromatin remodelling complexes modify the condensed DNA (chromatin) in a cell to allow access for transcriptional machinery. This opening and closing of chromatin can effectively switch genes on or off, thus altering the expression of genes through what is called epigenetics, rather than by changes in the DNA sequence (Figure 1).

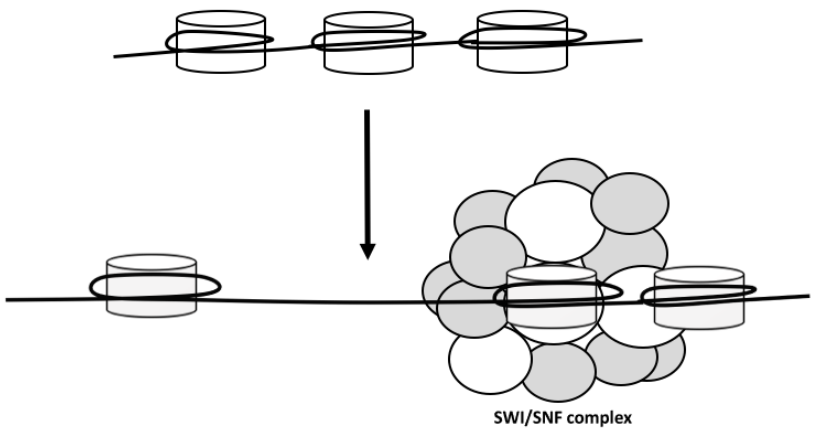

Figure 1. *Function of the SWI/SNF chromatin remodelling complex. The tightly wound DNA is opened and closed by the SWI/SNF chromatin remodelling complex, of which SMARCB1 and SMARCA4 are components. Chromatin modification in this way allows or denies access to the DNA for transcription factors, which are proteins involved in the regulation of gene expression. Mutation in SMARCB1 or SMARCA4 affects the ability of the complex to perform its functions, thereby altering the levels of expression of certain genes which in some cases leads to tumour development. Adapted from Wilson and Roberts (2011).*

SMARCB1 (also known as INI1, BAF47 or SNF5) is a gene that encodes the SMARCB1 protein, which is a subunit of the larger SWI/SNF chromatin remodelling complex (Roberts and Orkin, 2004). The genes encoding subunits of SWI/SNF complexes are particularly oncogenic, with mutations occurring in ~20% of all human cancers (Helming *et al.*, 2014). *SMARCB1* mutation can lead to loss of production of the SMARCB1 protein, which can result in interference of many important cell pathways. SMARCB1 mutation is a hallmark of rhabdoid tumours and is how they are genetically characterised, regardless of where they present in the body.

Many studies have established that SMARCB1 significantly influences MRT growth. In one study, 5 of 29 rhabdoid tumours from all recognised anatomic sites for MRTs had a biallelic deletion of the entire coding sequence for SMARCB1, a further 10 had a homozygous deletion of exon 1, and the remaining 14 were frameshift or nonsense mutations (Biegel *et al.*, 1999). In a similar study, biallelic inactivating events for SMARCB1 were found in 50 of 51 rhabdoid tumours (Jackson *et al.*, 2009). Thus, the notion that SMARCB1 is a tumour suppressor gene is strongly supported as its inactivation seems to be the single most important driver of rhabdoid tumour growth.

Lee *et al.* (2012) studied 32 frozen rhabdoid tumour samples and found that the genomes were incredibly simple, genomically stable and diploid and that loss of SMARCB1 was essentially the only recurrent event. In the case of 2 tumours, it was the only identified mutation. Another result of note from this study is that the mean mutation rate was just 0.19 mutations per DNA Mega base pair (Mbp). The research concluded that this is possibly the lowest rate of all currently-sequenced cancers, and particularly stands out because of the extremely aggressive and lethal nature of the cancer (Lee *et al.*, 2012).

Epigenetic changes are increasingly implicated in oncogenesis (McKenna and Roberts, 2009, Sparmann and van Lohuizen, 2006), however because most cancers are genomically unstable and have many mutations, the oncogenic effects of aberrant epigenetic influences is difficult to study and determine which (if any) epigenetic modifications are mainly responsible. In contrast, the simplicity of rhabdoid tumour genomes, along with most other SMARCB1-deficient cancers, allow rhabdoid cancers to be a useful model for further elucidation of the role of epigenetics in oncogenesis. It is highly likely that the role of SMARCB1 loss, leading to tumorigenesis is influenced by its role in epigenetic modification as a subunit of the SWI/SNF complex, however this requires further confirmation (Kim and Roberts, 2014).

In three separate studies the role of SMARCB1 in tumour growth was investigated using genetically engineered SMARCB1 knockout mice (Roberts *et al.*, 2000, Klochendler-Yeivin *et al.*, 2000, Guidi *et al.*, 2001). Homozygous inactivation of SMARCB1 leads to embryonic lethality, whereas heterozygous mice are born normally but are predisposed to cancer. Of the heterozygous mice, approximately 20% developed sarcomas with a median age of 12 months. While the location of these tumours are different to the sites of rhabdoid tumours in humans (all were extra-renal), there are clear comparisons to be made. The murine tumours closely resemble human rhabdoid tumours and include rhabdoid cells with standard human rhabdoid morphology. They were highly aggressive - as in humans , locally invasive, and often metastatic (Roberts and Orkin, 2004).

In other experiments, conditional biallelic SMARCB1 inactivation in mice results in a cancer predisposition and rapid aggressive tumorigenesis (Roberts *et al.*, 2002). All mice in this experiment developed tumours at a median age of

11 weeks. This pace of cancer development is incredibly rapid. For example, p53 deficiency results in cancer development at 20 weeks and p19ARF inactivation leads to cancer at 38 weeks. It is also interesting to note that reduced function or inactivation of SMARCB1 has been implicated in other cancers such as in familial schwannomatosis, a rare genetic disorder that can result in central nervous system tumours (Hulsebos *et al*., 2007), and epithelioid sarcoma, a rare sarcoma of soft tissue that frequently presents in the limbs of young adults (Hornick *et al*., 2009).

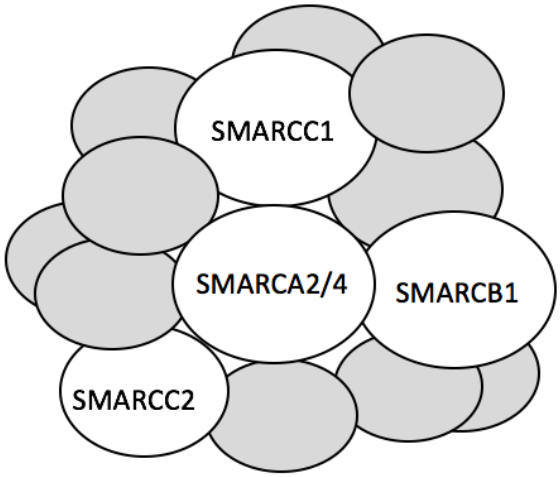

Figure 2. *An example of an SWI/SNF chromatin remodelling complex. Each SWI/SNF complex consists of an ATPase subunit (SMARCA4/SMARCA2), core subunits (SMARCB1, SMARCC1 and SMARCC2) and between 10-15 other accessory subunits (grey), enabling a vast number of possible combinations. SMARCB1 and SMARCA4 mutations have been directly linked to the proliferation of rhabdoid tumours. Adapted from Schneppenheim et al. (2010).*

SMARCA4 (also known as BRG1), the gene responsible for the expression of one of the two ATPase subunits in the SWI/SNF complex, has also been implicated in rhabdoid tumours (Figure 2). In a German study, it was found that two patients lacked SMARCA4 expression which suggests that SMARCA4 is also a tumour suppressor, and its inactivation can lead to rhabdoid tumours (Schneppenheim *et al*., 2010). This result is of note, because while another gene locus had been implicated in rhabdoid tumours it had not been identified in the literature previously (Frühwald *et al*., 2006). SMARCA4 inactivation has also been seen in small cell carcinoma of the ovary (SCCO), and it is thought that SCCO is consequently closely related to rhabdoid tumours (Ramos *et al*., 2014) or is a rhabdoid tumour itself (Fahiminiya *et al*., 2015). Rhabdoid tumour growth as a result of SMARCA4 mutations have been

linked to a higher rate of germline alterations than what is associated with SMARCB1 mutations, and affected individuals should seek genetic counselling (Hasselblatt *et al.*, 2014). Due to the potency of SMARCB1 as a tumour suppressor, significant efforts have been made to identify the cellular pathways affected by it and the mechanism through which it operates, although much is still unclear.

Pathways and Novel Therapies

Cyclin D1 and the Cell Cycle Pathway

Cyclin D1 is a critical sensor of extracellular stimuli and functions as a regulator of the cyclin-dependent kinases (CDKs). It was found in 2002 that re-introducing SMARCB1 to rhabdoid tumour cell lines induces cell cycle arrest by repressing cyclin D1 transcription via facilitation of histone deacetylase activity to the cyclin D1 promoter (Zhang *et al.*, 2002). Non-specific CDK inhibitors have been shown to inhibit rhabdoid tumour growth (Smith *et al.*, 2008). Mice which are SMARCB1 heterozygous and are lacking cyclin D1 do not produce rhabdoid tumours. This result provides clear *in vivo* evidence of cyclin D1 being at least one important factor for rhabdoid tumour growth, and that deletion of cyclin D1 is enough to block tumour development (Tsikitis *et al.*, 2005).

Pharmacological disruption of the cyclin D1 pathway is seen as a key potential target for future treatment. LEE011 is a small molecule inhibitor of CDK4/6 and is currently being used in a phase I clinical trial to investigate the effect inhibiting CDK4/6 has on patients (NCT02187783, 2016).

Sonic Hedgehog Pathway (SHH)

The SHH pathway plays an important role in differentiation during development. It is known to have a catalytic effect in oncogenesis. Loss-of-function mutations in the SHH pathway have been identified in medulloblastomas (a common paediatric brain cancer) and in familial and sporadic basal cell carcinomas (a common skin cancer) (Gailani *et al.*, 1996, Raffel *et al.*, 1997). GLI1 (glioma-associated oncogene homologue) is a transcription factor, making it involved in controlling the rate at which genes are read, and is a key mediator of the SHH pathway. The oncogenic potential of GLI1 has been shown in transgenic mice (Ruiz i Altaba *et al.*, 2007).

A link between SMARCB1 and GLI1 was investigated (Jagani *et al.*, 2010) and SMARCB1 and GLI1 were found to closely interact. This study also showed that when SMARCB1 is inactivated, GLI1 expression is increased eight- to tenfold. This result indicates that SMARCB1 plays a key role in repressing GLI signalling. Similarly SMARCA4 inactivation, and simultaneous inactivation of both SMARCA4 and SMARCB1 led to increased GLI1 expression, perhaps suggesting that GLI1 is a shared target for the SWI/SNF complex. GLI1 was upregulated in AT/RTs compared with control samples.

Arsenic trioxide (As_2O_3) targets GLI expression (Beauchamp *et al*., 2011), and it is an inhibitor of tumour cell growth of SHH-activated medulloblastoma (Kim *et al*., 2013). It is unknown how arsenic trioxide inhibits GLI expression, but it has been shown to be effective at inhibiting rhabdoid tumour cell growth both *in vitro* and *in vivo*. However, one concern with development of arsenic trioxide as a treatment for patients with rhabdoid tumours is the potential movement of the compound through the blood-brain barrier (Kerl *et al*., 2014).

Epigenetic antagonism between SWI/SNF and Polycomb complexes

Polycomb-group proteins (PcG) open or close chromatin and have well-known gene silencing abilities due to their epigenetic capability. Enhancer of Zeste Homolog 2 (EZH2) is the enzymatic component of the polycomb repressive complex 2 (PRC2), and it is overexpressed in many cancers with it being implicated in tumour progression (Chang and Hung, 2012). The apparent importance of EZH2 in oncogenesis has recently gained attention in and recent studies its role in cancer has been investigated (Suvà *et al*., 2009). It has been shown *in vitro* that polycomb proteins can directly repress the activity of the SWI/SNF complex (Shao *et al*., 1999). Inactivation of SMARCB1 leads to increased expression of EZH2 and its recruitment to polycomb targets. Thus, there appears to be epigenetic antagonistic properties between the SWI/SNF complex and PRC2. This observation was applied to an experiment wherein a mouse model's EZH2 gene was inactivated which thereby resulted in completely blocked tumour growth from SMARCB1 loss. This effect seems to be highly specific, as while the EZH2 loss resulted in tumour growth inhibition in the case of SMARCB1-deficient cancers, it had no effect on osteosarcomas resulting from p53/Rb loss (Wilson *et al*., 2010). In related studies, the efficacy of a small molecule EZH2 inhibitor called EPZ-6438 has been tested. EZH2-mutant xenograft-carrying mice models were given EPZ-6438 which was found to inhibit tumour growth and in some cases included complete tumour regression (Knutson *et al*., 2014). It was found that in the case of AT/RT, inhibiting EZH2 effectively sensitised rhabdoid tumour cells to radiation, identifying EZH2 as a potential therapeutic target (Alimova *et al*., 2013).

Aurora A

Aurora A (also referred to as Aurora Kinase A, STK6) is a mitotic gene and is often overexpressed in cancers, such as pancreatic and ovarian carcinomas (Wang *et al*., 2009), and this overexpression is also seen with SMARCB1-deficient rhabdoid tumours. Importantly, overexpression of Aurora A can transform normal cells (Katayama *et al*., 2003), which shows that it has strong evidence of being oncogenic. In another study (Lee *et al*., 2011), downmodulation of Aurora A resulted in a significant decrease in cell number and inhibition of cell proliferation, ultimately demonstrating that downmodulation of Aurora A is deleterious to rhabdoid

tumour cell growth. Alisertib (MLN8237) is a small, selective molecule inhibitor of Aurora A and due to strong results indicating anti-tumour activity preclinically (Maris *et al.*, 2010), a study was carried out to investigate its effect on rhabdoid tumours. Four patients with recurrent AT/RT were treated with alisertib and all four patients experienced disease stabilisation and/or decrease in tumour size after approximately 3-6 weeks. Two of the patients exhibited stable regression for 1 and 2 years. Alisertib had moderate toxicities including a decreased total WBC, neutropenia and thrombocytopenia (Wetmore *et al.*, 2015). Alisertib shows promise and is currently in phase II clinical trials (NCT02114229, 2015) .

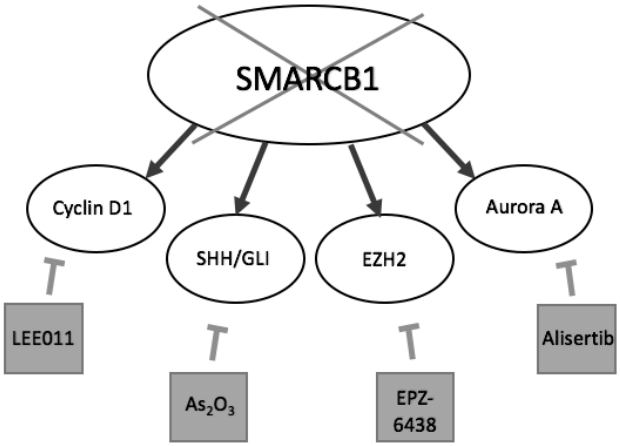

Figure 3. *Consequences of SMARCB1 mutations and therapeutic potential using inhibitors of affected pathways. Inactivation or loss-of-function of SMARCB1 upregulates the expression of many important pathway intermediary molecules such as cyclin D1, GLI1, EZH2 and Aurora A. Consequently, studies have investigated the efficacy of inhibiting these molecules with the aim of reducing tumour growth. Some of these targeted inhibitors which have shown potential as a treatment are shown here in grey.*

Conclusion

MRT is a highly aggressive cancer which mainly affects infants and young children. Inactivation or reduced expression of SMARCB1 is the main driver of its growth. Consequently, SMARCB1 has been identified as a critical tumour suppressor gene and many different pathways have shown to be affected by its inactivation. The results of some novel treatments targeting these pathways

are encouraging, such as the Aurora A inhibitor alisertib, the EZH2 inhibitor EPZ-6438, arsenic trioxide which targets GLI1 expression, the CDK4/6 inhibitor LEE011 and many more which are still in clinical trial phases (Figure 3). While rhabdoid tumours still remain devastating, the prognosis for patients with these tumours has improved in the last decade. The lessons that can be learned from the therapeutic approach to MRTs could have significant effects both for patient cohorts and for research efforts in finding a cure for malignant rhabdoid tumours.

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