Y-box binding protein-1 – a neglected target in pediatric brain tumors?

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Abstract

Brain and central nervous system (CNS) tumors represent the most common childhood solid tumors. Comprising 21% of all pediatric cancers, they remain the leading cause of cancer-related mortality and morbidity in childhood. Due to advances in neurosurgical technique, radiation therapy and the use of combination therapy, survival rates have generally increased. However, by cause of the lesion itself, its surgical removal and subsequent treatment, survivors are at high risk of long-term neurocognitive sequelae and secondary cancer. Clearly, improvements in diagnosis and treatment are needed. Accordingly, current treatment is evolving away from conventional, uniform therapy and towards risk-stratified regimens and molecularly-targeted therapies, with the aim of diminishing adverse side effects while minimising the risk of disease recurrence. The multi-functional oncoprotein Y-box binding protein 1 (YB-1) may serve as one such molecular target. Increased YB-1 levels have been reported in a number of pediatric brain tumors, where YB-1 appears to facilitate the advancement of malignant phenotypes. These include proliferation, invasion and resistance to therapy, as well as the maintenance of brain tumor initiating cells. Here we evaluate the current literature and show how YB-1 modulates signalling pathways driving each of these phenotypes. We also review the regulation of YB-1 at a transcriptional, translational, post-translational and sub-cellular level and argue that there is strong and sufficient evidence to support the development of YB-1 as a biomarker and future therapeutic target in childhood brain tumors.

Introduction

Cancer represents the leading disease-related cause of death in children residing in high-income countries. Brain and central nervous system (CNS) tumors are the most common solid childhood malignancies and the second most prevalent cancer in children after leukaemia, accounting for a quarter of all childhood cancers diagnosed each year in the UK. Considerable advances in imaging, neurosurgical technique, radiotherapy and the advent of combination chemotherapy means that survival rates for children with brain and CNS tumors has greatly improved over recent years. However, even with such advances, survival for children with brain and CNS tumors remains highly variable, with five-year survival ranging from less than 10% to over 90% depending on tumor type(1). Furthermore, long-term pediatric CNS tumor survival still remains lower than that of leukaemia and other solid pediatric malignancies(2). Additionally, research has shown that 80% of survivors go on to suffer significant, long-term consequences of exposing the developing brain to the aforementioned medical interventions including physical, cognitive, neurological and endocrine complications and an increased risk of secondary cancer(3). The long-term sequelae of treatment and the high rates of relapse observed in a number of pediatric brain tumor sub-types demands the development of alternative treatment strategies.

In recent years, advances in transcriptomics and DNA methylation profiling have transformed our understanding of the molecular underpinnings of a number of childhood brain tumors. Where tumors such as medulloblastoma were historically categorised into risk groups based upon histology, metastatic status and resection status, it is now recognised that these tumors encompass at least four distinct molecular sub-groups which differ genetically and phenotypically and are associated with different outcomes(4). Accordingly, at present, clinical trials are moving away from

treating brain tumors as a uniform disease and towards differential therapy for groups ascribed on the basis of molecular and clinical risk features.

Medulloblastoma

Medulloblastoma (MB), a malignant embryonal cerebellar neoplasm, is the most frequent brain tumor of childhood, comprising approximately 20% of all pediatric CNS tumor cases. Contemporary treatment protocols include maximal safe surgical resection with a combination of craniospinal radiotherapy and adjuvant chemotherapy(5). MB can occur at all ages, although the peak age of diagnosis is ~6 – 8 years and incidence declines with increasing age. Advances in molecular and genetic profiling uncovered substantial heterogeneity within MB and led to the identification of four core distinct molecular subgroups - Wingless (WNT), Sonic Hedgehog (SHH), Group 3 and Group 4 - which differ in their patient demographics, prognoses and metastatic status(4,6,7). More recently, on the basis of significant intra-subgroup heterogeneity, variances in age of onset and disparate prognoses, the existence of additional subtypes beyond the four consensus subgroups has been reported(8-11). Such variations, particularly in clinical response between both subgroups and subtypes, has promoted re-evaluation of patient risk stratification. Accordingly, it is now proposed that patients are stratified into low, standard, high, and very high clinical risk groups and treatment strategies altered to reflect this(12). Only one current clinical trial (NCT01878617) takes into account both subgroups and risk stratification. In this trial, low risk WNT patients, receive de-escalated treatment with the aim to reduce treatment-related morbidities, while skeletally mature standard- and high-risk SHH patients receive standard-of-care therapy with the addition of smoothened receptor inhibitors. Standard and high risk non-SHH/-WNT patients are then prioritised for intensified treatment.

Despite phenotypic and genetic variances, all MB tumors are classified by the World Health Organisation (WHO) as Grade IV (high-grade) neoplasms, owing to their aggressive and malignant behaviour(13). Indeed, a poor prognostic factor in nearly all MB patients (except those with WNT MB, where metastasis is rare) is the high frequency of metastasis, with up to 40% of pediatric patients exhibiting metastases at diagnosis and almost all patients metastatic at relapse(14). Indeed, survival rates of SHH, Group 3 and Group 4 patients exhibiting metastases at diagnosis range from under 50% to 75%, lower than that of non-metastatic patients which can range from 75% to over 90%(12). Patients with recurrent MB have poorer outcomes still, with a median survival of less than one year(15). Clearly, an improved understanding of the molecular events driving treatment resistance and recurrence represents a critical research area within the MB field.

Pediatric Glioma

Glioblastoma multiforme (GBM) is the most frequent and aggressive malignant primary brain tumor in adults. Accounting for 16% of all primary brain tumors, it is a deadly disease with a survival rate of just 14-15 months post-diagnosis, despite aggressive treatment. Likewise, pediatric GBM (pGBM) remains one of the few incurable pediatric cancers, with a long-term survival rate of less than 10%(16). Accordingly, it is classified as a WHO Grade IV tumor. Due to a lower incidence in children, studies have traditionally combined pGBM with anaplastic astrocytoma and diffuse intrinsic pontine glioma (DIPG), as high-grade gliomas. Together, these high-grade gliomas constitute 15% of pediatric CNS tumors.

Although pGBM are indistinguishable histologically from adult tumors, recent studies have considerably improved our understanding of the molecular biology both

separating and underlying adult and pediatric disease. In 2012, Sturm *et al.* identified six distinct GBM subgroups based upon global DNA methylation patterns: IDH, K27, G34, RTK I, RTK II (classic) and mesenchymal. While only the RTK II group is devoid of pediatric tumors, the majority of pGBM are found in the K27, G34 and RTKI subgroups where K27 and G34 are defined by mutations in *H3F3A* and RTKI by *PDGFRA* amplifications(17). Indeed, *PDGFRA* amplification, frequent chromosome 1q gain and less frequent chromosome 7 gain and 10q loss appear to represent markers of pediatric disease(18). An additional gene expression study performed specifically in pGBM revealed two molecularly diverse disease subsets (one with RAS and AKT pathway activation, one without) with differential survival within pediatric cohorts; importantly, these were also distinguishable at the molecular level from adult samples(19). Despite such significant variances in comparison to adult disease, current treatments for pGBM are predominantly based upon studies conducted in adults. As such, conventional therapy includes post-operative non-selective radiotherapy and chemotherapy(20). A better understanding of the molecular pathogenesis of pGBM is desperately needed in order to identify new targets for individualized or molecularly stratified therapies.

As previously described, pediatric high-grade gliomas encompass both anaplastic astrocytoma and DIPG in addition to pGBM. Representing approximately 10% of all pediatric CNS tumor cases, DIPG is a WHO grade IV tumor(13). Unlike other pediatric tumors, in which survival rates have improved substantially over the last four decades, outcome for DIPG patients remains dismal with a median survival of less than one year(21). This is largely due to the lack of therapeutic options available for treatment of this aggressive tumor. Recently, increased tumor tissue availability and next-generation sequencing methods has enabled the identification of two histone gene

aberrations – point mutations in *H3F3A* and *HIST1H3B*, encoding the histone variants H3.3 and H3.1. These mutations are present in ~80% of DIPG patients, differ in age and prognoses and importantly, may represent promising therapeutic targets(16). Anaplastic astrocytomas encompass 2% of all pediatric CNS tumor diagnoses and are categorised as WHO grade III tumors. Five-year survival for this malignant tumor is just 31%(22). Like pGBM, anaplastic astrocytomas are histologically indistinguishable from their adult counterparts, however exhibit significant differences at a molecular level. For example, where adult anaplastic astrocytomas commonly present with *EGFR* amplification and *IDH1* mutations, *H3F3A* mutations are common to pediatric disease ((22) and cited therein).

Ependymomas

Ependymomas are the third most common CNS tumor in children, accounting for 6 – 12% of cases. They can exist as WHO Grade I, II or III, although a number of studies have noted a lack of correlation between tumor grade and outcome(13,16). Despite recent advances in therapeutic approach, prognosis for this tumor type has remained relatively poor, particularly for infants in which five-year survival is just 42% - 55%(23). Additionally, one-third of ependymoma cases relapse, which like in MB, correlates with dismal prognosis(16). Historically, ependymomas were considered a single entity based on their morphological appearance. However, it is now clear that ependymomas arising within posterior fossa, supratentorial, and spinal anatomical locations represent distinct biological entities. As such, molecular profiling studies have further classified ependymomas into nine molecular subgroups – three in each anatomical compartment – to fully reflect the heterogeneity across and within compartments(23).

YB-1 in Brain Tumors – A Neglected Therapeutic Target?

The identification and development of molecularly-targeted therapy represents a critical area of pediatric neuro-oncology research, both to minimise adverse long-term sequelae associated with current regimens and to improve treatment efficacy, potentially reducing the rate of disease recurrence with its well-described dire prognosis. Y-box binding protein-1 (YB-1), encoded for by the *YBX1* gene on chromosome 1p34.2, may represent one such target. Although originally identified by its ability to bind specifically to Y-boxes (5'-CTGATTGGT/CT/CAA-3') in the promoters of target genes, it has since been demonstrated that YB-1 can interact with numerous RNA and DNA sequences and as such, YB-1 is now considered a multi-functional protein with extensive roles beyond its transcription factor functionality(24). In fact, YB-1 has been implicated in almost all DNA- and mRNA-dependent processes within the cell, including mRNA translation and packaging, DNA repair, proliferation, pre-mRNA splicing and DNA replication(25).

Due to the multi-functional nature of YB-1 and its position upstream of numerous cellular signalling pathways, including E2F, PI3K/AKT/mTOR, RAS/RAF/MEK/ERK and P53 pathways, (discussed extensively in (26)), it is perhaps not surprising that YB-1 has been heavily linked to cancer. Over-expression has been recorded in numerous tumor types including breast cancer, osteosarcoma, cervical cancer and lung cancer where expression, and in particular nuclear expression, correlates with poor prognosis, proliferative and metastatic potential and drug resistance((27) and cited therein).

Despite considerable research in solid tumors, there is a significant lack of studies exploring the role of YB-1 in pediatric brain tumors or indeed in brain and CNS tumors

in general. Elevated *YBX1* expression has been reported in both pGBM and MB(19,28,29). Further, analysis of large-scale, publically available datasets shows *YBX1* expression is substantially increased not only in MB and pGBM, but also in DIPG, anaplastic astrocytoma and ependymoma (Figure 1A). In both MB and pediatric high-grade glioma, elevated *YBX1* expression correlates with poor survival outcome (Figure 1B-C). The high expression and prognostic significance of YB-1 in pediatric brain tumors therefore suggests that it may represent a clinically relevant target worthy of further investigation. In this review, we discuss YB-1 structure and regulation and explore how its diverse cellular functions may facilitate disease progression in pediatric brain tumors.

Structural Organisation of YB-1

YB-1 is a 324 amino acid protein with a molecular mass of 35.9 kDa (Figure 2). It comprises of three domains: a short alanine/proline-rich N-terminal domain (A/P domain), a highly conserved cold shock domain (CSD), and an elongated C-terminal domain (CTD) with alternating clusters of positively and negatively charged amino acid residues(30). The three-dimensional structure of the CSD domain has been resolved by NMR and is known to adopt a classical oligonucleotide/oligosaccharide (OB)-fold consisting of a closed five-stranded anti-parallel β-barrel(31). The CSD contains ribonucleoprotein (RNP)-1 and RNP-2 RNA binding motifs and is responsible for both specific and non-specific RNA binding and specific ssDNA binding. Recently, it was demonstrated that the YB-1 CSD forms a homodimer, both in a crystal structure as well as in solution. This has been proposed to facilitate the RNA binding ability of YB-

1 and its multimerisation through the CTD - a process involved in mRNA packaging and translational control(32).

Contrastingly, the 3D structures of both the CTD and A/P domains are unknown, likely due to their predicted intrinsically disordered nature(33). The A/P domain contains binding sites for various proteins, including serine/arginine rich splicing factor 9 (SRP30C) (also interacts with the CSD), cyclin D1, actin and P53 (34-37). Likewise, the CTD has been reported to bind a number of proteins, including P53, DNA repair protein NEIL2 and YB-1 itself (38-41). The CTD also contains several proposed nuclear localisation signals (NLS), all of which are denoted in Figure 2. Originally, the YB-1 NLS was identified at residues 183 – 202(42). More recently, a further set of NLS have been proposed: NLS-1 at residues 149 – 156, NLS-2 at residues 185 - 194 and NLS-3 at residues 276 – 292(43). Finally, a proline-tyrosine(PY)-NLS, was identified at residues 174 – 202, closely matching the position of the originally identified NLS(44). Two cytoplasmic retention sites (CRS) have also been suggested, residing at residues 247 – 267 and 264 – 290(42,45). The CTD also has nucleic acid binding capabilities. While the CSD appears to have a more significant role in RNA binding, it is thought that DNA binds with highest affinity to the CTD(46).

YB-1 Regulation – An Intricate Web of Transcriptional, Translational and Post-Translational Control

Regulation of YB-1 Expression

In spite of interest in YB-1 and its functions, our understanding of the regulation of *YBX1* expression remains vague. Several transcription factors have been shown to regulate *YBX1* through interaction with a number of different motifs throughout both

the *YBX1* promoter and 5'untranslated (UTR) region (Table 1). Correspondingly, control of *YBX1* expression is likely to be highly context-dependent and may be triggered in response to various stimuli. Six E-boxes are located throughout the *YBX1* promoter. E-box binding transcription factor C-MYC has been shown to interact with the *YBX1* promoter and drive transcription in a P73-dependent manner in response to DNA-damaging stimuli(47). Additionally, basic-helix-loop-helix transcription factor TWIST1 has been implicated in E-box dependent *YBX1* transcriptional control(48), a finding further validated by the involvement of TWIST1 binding proteins p300/CBP-associated factor (PCAF) in *YBX1* transcriptional activation and Programmed Cell Death Protein 4 (PCDP4) in *YBX1* down-regulation(49,50). A 5'UTR GGATAA element has also been shown to activate the YB-1 gene promoter through binding of both GATA-1 and GATA-2 to this region, although interestingly not to GATA elements present within the promoter(51).

Post-transcriptional regulatory mechanisms of YB-1 are better described (Table 1). YB-1 translation is controlled by an auto-regulatory feedback loop in which YB-1 binds to a ~80-nucleotide regulatory element in the 3'UTR of its own mRNA, which encompasses overlapping binding sites for both YB-1 and poly(A)-binding protein (PABP). PABP binds to an 50-nucleotide A-rich sequence within the regulatory element, stimulating *YBX1* mRNA translation in a poly(A) tail-independent manner. Conversely, YB-1 selectively inhibits its own synthesis through interaction with two sequences within the regulatory element, both containing the same 8-nucleotide motif - UCCAG/ACAA. The binding sites of YB-1 and PABP overlap, meaning that the two proteins compete for *YBX1* mRNA binding(40,52). YB-1 is a major protein in both polysomal and free messenger RNPs (mRNPs) and plays an extensive role in the regulation of overall protein synthesis within the cell, depending on the amount of YB-

1 associated with mRNA. In the complete absence of YB-1, or conversely at high YB-1 concentrations where mRNA is saturated with YB-1, translation is suppressed; whereas at relatively low YB-1 levels, translation is stimulated(25). Thus, YB-1 appears to auto-regulate its own synthesis at a concentration that is optimal for the translation of other cellular mRNAs.

Moreover, YB-1 translation also appears to be sensitive to signalling through the mTOR pathway, dictated by sequences in the 5'UTR of *YBX1* mRNA. Regulation in this way appears to be highly dependent on cell division rate. When cells are slow-dividing/serum starved, mTOR signalling is attenuated and hence YB-1 translation is inhibited(53). Various families of non-coding RNA (ncRNA) have also been implicated in the regulation of *YBX1* expression, including miR-137 and miR-216a, the lncRNA GAS5 and MIR22HG, reviewed in(54).

YB-1 is known to undergo various post-translational modifications (Table 1; Figure 2), with phosphorylation being the focus of the majority of research to date. Indeed, there is evidence to suggest that phosphorylation "activates" YB-1, promoting both nuclear transport and facilitating DNA binding. The most extensively studied phosphorylation site in YB-1 is serine-102 (S102) in the CSD, which kinases AKT and RSK have been shown to target, implicating both the MAP kinase pathway and PI3K cascade in the control of YB-1 activation(55,56). Other confirmed phosphorylation sites include: S165 (CSD), which appears to be crucial for transcriptional activation of nuclear factor kappa (NF-κB); tyrosine-162 (Y162) (CTD), phosphorylation of which is mediated by fibroblast growth factor receptor 1 (FGFR1) and Y188 and Y281 (CTD), where Y281 phosphorylation appears to correlate with YB-1 nuclear shuttling(57). Acetylation sites have also been reported, including lysine-81 (K81) in the CSD as well as K301 and K304 in the CTD. Although the functional significance of K81 acetylation is unknown,

acetylation at positions K301 and K304 has been suggested to promote microvesicle-mediated secretion of YB-1 from the cell(57,58). Ubiquitination sites have also been identified in YB-1 and retinoblastoma binding protein 6 (RBBP6), an E3 ubiquitin ligase, has been shown to interact with YB-1, leading to ubiquitination and proteasomal degradation(57). Additionally, the peptide bond linkage between glutamate-219 (Glu219) and glycine-220 (Gly220) has been reported to be targeted by the 20S proteasome for endoproteolytic cleavage in an ubiquitin-independent manner and may also be involved in YB-1 nuclear shuttling(43,59).

Regulation of YB-1 Cellular Localisation

YB-1 is a cytoplasmic-nuclear shuttling protein. In non-malignant cells, over 90% of YB-1 is located in the cytoplasm where it is associated mRNPs. The NLS and CRS located in the CTD of YB-1 appear to regulate YB-1 distribution between the nucleus and the cytoplasm. The CRS is thought to be dominant, which combined with the affinity of YB-1 to mRNA and its interaction with cytoplasmic partner proteins, is responsible for YB-1 cytoplasmic retention(39). The exact mechanism of YB-1 translocation to the nucleus is not fully understood (Figure 3) and appears to be a dynamic process that occurs on account of YB-1 being able to undergo structural rearrangements in response to various stimuli, resulting in exposure of NLS to transport proteins. Indeed, transportin-1 has been proposed to mediate YB-1 nuclear import through interaction with the PY-NLS(44).

It has been suggested that changes in YB-1 nuclear localisation occur in a cell cycle-dependent manner, with nuclear accumulation observed during the G1/S transition. Two regions in the CTD, encompassing NLS-2 and NLS-3, were shown to mediate translocation in this context (60). YB-1 subcellular localisation may also be mediated,

in part, by interaction with other proteins. YB-1 is known to play a role in pre-mRNA splicing, however, YB-1 appears to interact directly with splicing factor SRP30C via the A/P domain in an RNA-independent manner. Co-localisation of YB-1 and SRP30C has been shown to promote significant nuclear shuttling of YB-1, an effect which is reversed upon sequestering of SRP30C into stress-induced nuclear bodies following heat-shock(35). Another study, undertaken in breast cancer cells, highlighted the importance of WAVE3, a member of the WASP/WAVE actin-cytoskeleton remodelling protein family. The WAVE3-YB-1 interaction appears to be facilitate the nuclear translocation of YB-1, which in this context promoted the transcriptional regulation of cancer stem cell (CSC)-specific genes implicated in self-renewal and expansion(61).

Clear evidence exists for YB-1 nuclear translocation in response to cellular stress, for example upon exposure to UV radiation, oxidative stress, hyperthermia and chemotherapeutics((62,63) and literature cited therein). A 2005 study revealed that wild-type, transcriptionally active P53 is necessary for efficient nuclear translocation of YB-1, likely via the trans-activation of a P53 target 'effector' gene(64). Comparatively, a 2006 study suggested that YB-1 accumulates in the nucleus by complexing directly with P53 and WRN, a process thought to facilitate WRN-mediated DNA repair(65). As previously described (See Regulation of YB-1 Expression), proteolytic cleavage between Glu219 and Gly220 by the 20S-proteasome under cellular stress may also trigger YB-1 nuclear localisation through the production of a fragmented YB-1 protein lacking a CRS(43,59). However, the proteasomal theory of YB-1 nuclear accumulation is still disputed, largely due to the lack of a suitable YB-1 antibody (See YB-1 as a Biomarker)(45,66).

Lastly, RSK1 and RSK2 have been shown to directly phosphorylate YB-1 at S102, with knockdown/drug-mediated inhibition diminishing YB-1 promoter binding(55).

Experiments using breast and ovarian cancer cell lines have shown phosphorylation by AKT at S102 promotes nuclear translocation; disruption of which, either by mutation or inhibition, prevents nuclear localisation and negatively affects cell growth and the expression of drug resistance genes(56,67). Contrastingly, some groups have found no evidence of AKT-mediated phosphorylation at S102 influencing cellular localisation at all, with mutation of the S102 site resulting in unchanged nuclear/cytoplasmic distribution of YB-1 ((68)and studies cited therein). Alternatively, phosphorylation at Y281 within NLS-3 may act dominantly on the subcellular localization of YB-1, promoting nearly exclusive nuclear localization of full-length YB-1(43). Additionally, a recent report proposed that rather than cytoplasmic RSK promoting the phosphorylation and resultant nuclear shuttling of YB-1, nuclear-phosphorylated YB-1 (pYB-1) is instead a product of the nuclear translocation of RSK, which then phosphorylates and activates pre-existing nuclear YB-1 in response to cellular stress(69).

YB-1 – A Master Regulator of Cancer Cell Biology

YB-1 and Proliferation

Sustaining proliferative signalling via dysregulation of the cell cycle is arguably the most important hallmark of a tumor cell. Reduction of YB-1 results in diminished proliferation and apoptosis in cancer cell lines and *in vivo* models, with a number of studies revealing a reduction in cyclin and cyclin-dependent kinase (CDK) level upon YB-1 knockdown(60,67,70,71). In MB, YB-1 appears to be critical for sustaining proliferation both in cells and tissues derived from SHH MB mouse models and in cerebellar granule neuron precursors (CGNPs; the proposed cells-of-origin for SHH

MB) *in vitro* and *ex vivo*(28). Likewise, a study examining the oncogenic functions of YB-1 in pGBM revealed silencing of YB-1 significantly reduced SF188 cell growth in monolayer and soft agar and delayed tumor growth in mice(72).

Several pathways that promote cancer cell proliferation are activated by YB-1, including E2F, PI3K/AKT/MTOR and RAS/RAF/MEK/ERK(26). A prime example is the activational role played by YB-1 in the regulation of *IGF2* expression in CGNPs and medulloblastoma cells (Figure 4A)(28). The Insulin-like Growth Factor (IGF) signalling pathway has been reported in MB, is required for SHH MB formation and MB proliferation control and has been associated with metastatic progression(14,73). For example, activation of the IGF receptor by IGF1/2 results in downstream activation of PI3K signalling, leading to inhibition of GSK3β, a kinase responsible for blocking cell cycle progression in CGNPs(28,73). Interestingly, in the aforementioned study, YB-1 was shown to be induced by Sonic Hedgehog (Shh) in CGNPs, demonstrating co-operation between SHH and IGF-mediated PI3K signalling and identifying the SHH:YB-1:IGF2 axis as a powerful target for therapeutic intervention in medulloblastoma. Furthermore, a 2007 study assessing molecular pathways in pGBM identified at least two disease subsets, one poor prognosis associated with a proliferative phenotype and RAS and AKT pathway activation and one good prognosis without. Of particular interest, the subset with RAS and AKT pathway activation exhibited nuclear YB-1 expression which was associated with elevated *EGFR* expression, while the good prognosis subset exhibited predominantly cytoplasmic YB-1 expression (Figure 4A). Thus, in pGBM YB-1 may undergo AKT-mediated phosphorylation, resulting in nuclear translocation (Figure 3) and concordant transcription factor functionality while relieving translational repression of numerous pro-mRNAs, hence contributing to increased *EGFR* levels, RAS activity and

gliomagenesis(19). A similar finding was reported in a lung cancer study, where nuclear YB-1 localisation was associated with *EGFR* expression and poor prognosis(74).

YB-1 and Invasion/Metastasis

Metastasis is the leading cause of cancer mortality. At the point of diagnosis up to 40% of MB patients and 17% of pGBM patients display clinically detectable metastatic disease(14,75). Metastasis is a complex process requiring a number of events which can vary between cancer types, however common to all are changes in cell-cell and cell matrix adhesion, cell polarity and cytoskeletal organisation. A number of studies have revealed a clear role for YB-1 in invasion and metastasis. Bioinformatic analysis of a large breast cancer micro-array displayed a strong association between *YBX1* mRNA expression and distant metastasis formation(76). *In vitro* studies have demonstrated that YB-1 depletion impedes the invasive capabilities of a number of cancer cell lines(77,78). Likewise, knockdown of YB-1 both stably and transiently in pGBM cell line SF188 significantly reduces cell invasion in transwell invasion assays (Figure 4B)(72). The mechanisms underlying this process have not been studied in pGBM, perhaps owing to the fact that the molecular pathways involved in metastasis in pediatric brain tumors remain largely unclear. However, in other cancer types YB-1 has been shown to regulate multiple stages of the metastatic cascade at the level of transcription, pre-mRNA splicing and translation.

One of the first steps in metastatic dissemination is loss of polarity and cellular adhesion within the primary tumor mass. A key step in the loss of adhesion in epithelial cells is the inactivation of adherens junction protein E-cadherin by epithelial to mesenchymal (EMT)-inducing transcription factors, such as SNAI1 and TWIST.

Although only recently has the EMT process in non-epithelial tumors been considered important for tumor progression, contemporary glioma research has named the EMT a key player in glioma invasion, with “mesenchymal” a known GBM subtype associated with poor outcome(79). Interestingly, malignant CNS tumors frequently exhibit minimal E-cadherin expression, with one study demonstrating a marked reduction in E-cadherin in adult and pediatric high-grade glioma and medulloblastoma compared to low-grade gliomas, perhaps indicative of the highly aggressive, malignant nature of these tumors(80). Gain- and loss-of-function studies in cervical and breast cancer lines have revealed that YB-1 plays a key role in the translational regulation of proteins implicated in the acquisition of a migratory mesenchymal phenotype including SNAI1/2, ZEB2 and TWIST1(81,82). Correspondingly, over-expression of YB-1 in breast cancer cells is associated with loss of E-Cadherin and tight junction protein ZO-1, increased expression of N-cadherin and vimentin and the emergence of a mesenchymal phenotype(81). As both SNAI1 and ZEB2 have been associated with invasion, migration and EMT in pediatric and adult glioma and glioblastoma (Figure 4B), studying the role of YB-1 in this context in brain tumors may yield interesting results(83,84).

The basement membrane (BM) and extracellular matrix (ECM) represent a substantial physical barrier to the migration of cancer cells. Basement membranes in the CNS include: the pial basement membrane, the vascular basement membrane and the basement membranes that are associated with Schwann cells. Both medulloblastoma and pGBM can disseminate through passive shedding into the cerebral spinal fluid (CSF) system. Although the mechanism by which this occurs is not fully understood, in order for invading cells to extend into the CSF they would have to cross one or more CNS basement membranes. To do this, multiple proteolysis pathways are likely to

become activated. Studies have shown YB-1 to regulate the expression of numerous matrix metalloproteinases. YB-1 knockdown in pancreatic and melanoma cancer cell lines results in significant down-regulation of MMP-11, MMP-14 and MMP-2(77,85). Moreover, in ER-positive breast cancer cells, YB-1 appears to enhance the presentation of membrane type-1 (MT1)-MMP at the sites of cell invasion, namely the cell membrane, where it can degrade the ECM(86). MMPs have been shown to be associated with glioblastoma and medulloblastoma invasiveness (Figure 4B). In particular, (MT1)–MMP expression is increased in >75% of medulloblastoma tumor samples and MMP2 expression is associated with pediatric high-grade gliomas(87,88).

Further, YB-1 induces transcriptional activation of CD44, a non-kinase transmembrane glycoprotein over-expressed in cancer stem cells and implicated in tumor cell invasion and drug resistance(89). YB-1 can also stimulate alternative splicing of CD44 pre-mRNA resulting in exon v4 inclusion and the production of CD44v4. The CD44v4 variant has been shown to promote both cancer cell invasion and chemoresistance in various cancer cell lines(90,91). Although yet to be researched in specifically pGBM, increased CD44 and CD44 variant expression has been described in GBM (particularly in the mesenchymal subgroup), where expression is associated with poor survival (Figure 4B). *In vitro*, the extracellular domain of CD44 appears to facilitate GBM cell invasion through direct interaction with hyaluronic acid and other matrix factors, promoting cell motility through ECM adherence(92).

YB-1 and Multi-Drug Resistance

The development and acquisition of multiple drug resistance (MDR) in cancer cells remains a major obstacle in the treatment of metastatic disease. YB-1 knockdown has

been shown to increase cellular sensitivity to numerous cytotoxic drugs including cisplatin and etoposide (85,93). Correspondingly, YB-1 knockdown in pGBM SF188 cells, as well as GBM U251 cells renders cells sensitive to both temozolomide and taxol in an O^6-methylguanine-DNA methyltransferase (MGMT)-independent manner(72). As previously mentioned (See Regulation of YB-1 Subcellular Localisation), exposure of various epithelial cancer lines to UV radiation, oxidative stress, hyperthermia and chemotherapeutics promotes YB-1 nuclear localisation ((62,63) and literature cited therein). YB-1 nuclear translocation has also been recorded in Group 3 and SHH MB cell lines in response to treatment with cisplatin and vincristine (unpublished observations; Louisa Taylor, Ian Kerr and Beth Coyle). Moreover, in brain tumor initiating cell (BTIC) lines derived from patients with primary GBM, YB-1 is highly phosphorylated and localised to the nucleus in temozolomide-resistant lines, whereas in lines with low resistance, YB-1 is predominately located in the cytoplasm(94). Such observations indicate that cancer cells may increase nuclear YB-1 expression/promote nuclear YB-1 translocation as a protective measure in response to extracellular stress.

Perhaps the best known mechanism for the development of MDR is the enhanced synthesis of certain members of the ATP-binding cassette family, most notably ABCB1 (P-glycoprotein). ABCB1 has been implicated in the cellular export of a wide range of chemotherapeutics including vinka alkaloids, anthracyclines, taxanes and protein kinase inhibitors. Of particular note, a significant correlation between ABCB1 expression and high-risk MB has been demonstrated, indicating that overcoming/inhibiting ABCB1 may represent a mechanism to enhance the efficacy of current chemotherapy regimens in MB(95). Indeed, correlation between ABCB1 expression and nuclear YB-1 translocation has been demonstrated in breast cancer

patients treated with paclitaxel and in prostate cancer patients following neo-adjuvant hormone therapy(62,96). Early studies suggested YB-1 may transcriptionally regulate *ABCB1* expression by interacting with a Y-box element present in the *ABCB1* promoter region in response to cellular stress (Figure 4C)(63,97). Controversially, other studies have disputed this. A 2016 study using triple-negative breast cancer lines demonstrated that although high YB-1 expression correlates with increased resistance, this occurs in an ABCB1-independent manner(98). Likewise, others have argued that there exists more evidence to support nuclear factor Y (NF-Y) as a Y-box element-binding transcription factor in cancer cells, suggesting a post-transcriptional role for YB-1 in the regulation of *ABCB1*/other Y-box element containing genes(99).

Another mechanism of resistance to cytotoxic therapy is the activation of DNA repair mechanisms and the evasion of drug induced apoptosis. YB-1 has long been suggested to be part of the DNA repair machinery (Figure 4C). YB-1 possesses high affinity to cisplatin-modified DNA and DNA containing abasic sites/mismatches and possesses intrinsic exo- and endo-nuclease activity. It also interacts with several key components of base- and nucleotide-excision repair pathways (reviewed in (25)). Such observations, combined with observed YB-1 nuclear translocation in response to cytotoxic therapy, supports the theory that YB-1 is involved in the repair of DNA lesions imparted by genotoxic therapy, conferring drug resistance to tumor cells. At the same time, YB-1 has been proposed to regulate key pro-apoptotic and immune response genes including *FAS*, *MHC Class I* and *II* and notably key tumor suppressor gene *TP53*. Indeed, *YBX1* knockdown in SHH MB cell lines promotes apoptosis through the de-regulation of heterochromatin-regulated genes associated with inflammatory response, apoptosis and death receptor signalling. This occurs through a concurrent reduction in CDX5 expression, a heterochromatin-associated protein regulated post-

transcriptionally by YB-1, which represses transcription of apoptosis-related genes through Histone 3 K9 trimethylation (H3K9me3) interaction(29).

Perhaps counterintuitively given the role of P53 in YB-1 nuclear translocation (Regulation of YB-1 Cellular Localisation), YB-1 can both repress transcription of *TP53* and inhibit the ability of P53 to transactivate cell death genes *BAX* (Bcl2-associated X protein) and *NOXA* (NADPH oxidase activator); although interestingly not cell cycle-associated gene *CDKN1A* (Cyclin Dependent Kinase Inhibitor 1A)(64,70). The mechanism by which this occurs likely requires direct interaction between P53 and YB-1, reducing its affinity for promoter binding and causing promoters with a low binding affinity for P53 (i.e. pro-apoptotic genes) to be disabled(64). More recently, over-expression of YB-1 in GBM cell lines U87 and DK-MG was shown to promote temozolomide resistance through direct interaction with the MDM2/P53 signalling pathway. YB-1 over-expression results in MDM2 activation and the resultant ubiquitination and proteasomal degradation of P53, inhibiting P53-mediated apoptosis (Figure 4C)(100). In support of this, overexpression of MDM2 was recorded in 67% of patients in a pediatric high grade astrocytoma cohort, with P53 tumor suppressor pathway inactivation, either by single or combinatorial events taking place in >95% cases(101). Furthermore, *in vivo* studies have highlighted a role played by MDM2 in connecting SHH and P53 pathways in CGNPs, suggesting that MDM2 may be required for SHH medulloblastoma tumorigenesis(102). Notably, SHH patients harbouring germline *TP53* mutations represent a very high risk group in medulloblastoma, with survival rates of less than 50%; further highlighting the importance of P53 pathway disruption in medulloblastoma. Taken together, targeting genes implicated in the P53 tumor suppressor pathway, such as *YBX1,* is likely to be of high therapeutic value for the treatment of pediatric brain tumors.

YB-1 and Brain Tumor Initiating Cells (BTICs)

As previously described, frequent relapse represents a serious obstacle to pediatric brain tumor survival. A number of factors are associated with poor responses to therapy and concurrent relapse, including the presence of BTICs, referred to in some studies as brain cancer stem cells. BTICs are multipotent, have the ability to self-renew, form neurospheres and initiate tumor development(103). YB-1 was first associated with TICs in 2010, where it was reported to induce breast TICs to express CD44 and CD49f, leading to enhanced cell growth and drug resistance(89). Both PI3K/AKT and RAS/MAPK pathways are activated in BTICs, in which YB-1 is a downstream phosphorylation substrate. Correspondingly, in BTIC lines derived from primary GBM patients, both total and phosphorylated YB-1 was highly elevated compared to normal CNS tissue(94). This is supported by a comprehensive GBM study by Fotovati et al(103). YB-1 was found to co-localise with neural stem cell markers Nestin, SOX2 and BMI-1 in SF188 pGBM cells cultured as neurospheres, as well as GBM patient derived BTIC isolates (Figure 4D). YB-1 knockdown was associated with loss of neural stem cell markers, reduced proliferation and differentiation in SF188 neurospheres, while forced differentiation of primary BTICs resulted in loss of YB-1 expression. Importantly, high and co-ordinated expression of YB-1, SOX2 and BMI-1 was detected in 67% of GBM cases which subsequently went on to relapse(103). Consistent with these observations, a recent study which employed CRISPR/Cas9 to knockout YB-1 in melanoma and breast TICs revealed that that YB-1 maintains the stemness of TICs by promoting the expression of stemness-related genes *FZP-1*, *GLP-1*, *GINS1* and *NOTCH2*(104). Collectively, these studies imply that targeting YB-1 will stimulate BTICs to undergo differentiation and

supress their proliferative capacity and hence further supports the development of YB-1 targeted therapies as a novel approach in the management of aggressive pGBM.

YB-1 and Pediatric Brain Tumors – Clinical Relevance

YB-1 as a Biomarker

There is evidence to support the use of YB-1 as a prognostic biomarker in pediatric brain tumors. Analysis of patient data demonstrates that *YBX1* mRNA is up-regulated in MB and high-grade pediatric glioma where it correlates with poor survival outcome (Figure 1A-C)(19,28,29). Indeed, a number of studies have proposed YB-1 as a novel biomarker of glioma progression. In GBM patient samples, YB-1 protein expression has been shown to increase with tumor stage(103). Likewise, in a larger study, YB-1 protein/mRNA levels were shown to differ significantly between tumor grades, with Grade I/II tumors presenting with mainly cytoplasmic staining and Grade III/IV presenting with abundant nuclear and cytoplasmic staining, which was found correlate with poor overall survival(105). Of particular note, YB-1 was significantly elevated in the CSF of Grade III/IV patients compared to that of Grade I/II patients, indicating that YB-1 may also represent a promising CSF marker for distinguishing malignant gliomas(105). The aforementioned studies demonstrated that overall YB-1 over-expression is associated with poor prognosis, however it may also be important to consider YB-1 subcellular localisation when determining the prognostic value of YB-1 expression. For example, although YB-1 was found to be up-regulated in 86% patient samples in a pGBM cohort, high nuclear expression was associated with an AKT-active poor prognosis subgroup, whereas samples exhibiting predominantly

cytoplasmic expression were associated with a better prognosis, AKT-inactive subgroup(19).

A potential barrier to the use of YB-1 as a prognostic marker in pediatric brain tumors, or indeed in other cancer types in the clinic, has been the variability in available antibodies. As previously reported, there exists controversy surrounding the function of proteolysis in the regulation of YB-1 nuclear translocation (See Regulation of Subcellular Localisation), arising from cross-reaction between N-terminal YB-1 antibodies and another protein called heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1)(66). The hnRNPA1 protein is present in both nuclear and cytoplasmic compartments and migrates at ~37 kDa (YB-1 migrates at ~49 kDa), meaning immunostaining products produced by such cross-reaction are very difficult to interpret(45,66). Despite this issue being raised by research and review articles alike, one commercially available N-terminal cross-reactive antibody (abcam; ab12148) continues to be used, with 9 references in 2019 alone(26,45,54,66). Further to this, disparities have also been demonstrated in the ability of YB-1 antibodies to detect nuclear YB-1 protein in addition to cytoplasmic YB-1, a finding that may prove important if the quantification of nuclear expression specifically is required to assess prognosis(45). Standardisation of one properly validated antibody is of fundamental importance in order to develop effective prognostic screens using YB-1 as a biomarker.

YB-1 as a therapeutic target

Given the multi-functional nature of YB-1 and its position upstream of numerous tumor-promoting molecular pathways, YB-1 represents an attractive therapeutic target. One potential therapeutic approach to directly target YB-1 is through oligonucleotide-based

methods including siRNA and miRNA. YB-1 knockdown by siRNA has proven effective *in vitro,* resulting in reduced proliferative and migratory capability and increased drug sensitivity in pediatric brain tumor cells(72,103). However, the delivery of siRNA to tumor cells in the human body, especially those metastasised, represents a major challenge to using such molecules in the clinic. Unmodified siRNA is rapidly degraded in the bloodstream, does not readily enter cells and can be immunogenic. The blood-brain barrier (BBB), comprised of a tight arrangement of endothelial cells, represents a further challenge for efficient siRNA delivery. There are a number of systems which may represent viable options to deliver oligonucleotide-based therapy to brain tumors, including lipid-based, inorganic and polymeric nanoparticles. Poly(β-amino ester) (PBAE)-based nanoparticles have successfully been used to deliver and efficiently release siRNA in a patient-derived GBM mouse model, successfully knocking down a reporter gene within GBM cells without systemic toxicity(106). Dual-modified cationic liposome nanoparticles incorporating CD133-targeting ligands and encompassing paclitaxel and survivin siRNA have also proven successful in *in vitro* and *in vivo* glioma studies, displaying high specificity for CD133+ glioma stem cells and low toxicity to brain endothelial cells(107). As an alternative to larger nanoparticles, which frequently accumulate in the reticuloendothelial system of the liver and spleen, a recent study proposed an alternative oligonucleotide stabilisation approach using Y-shaped block catiomers (YBCs). Notably, the number of positive charges in the YBC can be adjusted to match that of negative charges in each oligonucleotide strand, facilitating selective paring in the bloodstream. The resultant complex is stable in the blood stream and incredibly small (~18nm), allowing efficient delivery in an *in vivo* patient-derived GBM model(108). Although further pre-clinical and clinical trials are required, such studies

show oligonucleotide-based therapy, both by nanoparticles and alternative approaches, to be a promising brain tumor treatment strategy.

A number of groups have proposed that the transcription factor functionality of YB-1 is activated by phosphorylation. For this reason, disruption of YB-1 phosphorylation may represent another approach to targeting YB-1 activity. The most widely characterised phosphorylation site in YB-1 is S102 (Figure 2). Blocking the S102 site by way of a decoy cell permeable peptide led to inhibition of *EGFR* expression and reduced growth in prostate and breast cancer cell lines, without affecting non-malignant cells(109). Upstream inhibitors targeting RSK and AKT may also be an option. Fisetin is a dietary flavonoid which is able to bind directly with RSK, both suppressing RSK kinase ability and promoting interaction between fisetin and the YB-1 CSD. Together, this led to a decrease in YB-1 phosphorylation and down regulation of total YB-1 protein, with a concurrent decrease in MMP-2, MMP-9 and ABCB1 expression and reduced cell viability in a melanoma model(110). However, it must be noted that some groups have found no evidence of YB-1 phosphorylation influencing nuclear translocation (See Regulation of Subcellular Localisation). Further study of the validity of the phosphorylation theory of YB-1 nuclear localisation and as well as in which cancer types/cell types this theory holds true will be required prior to the development of this therapeutic approach.

Over recent years virotherapy has emerged as an alternative treatment for cancer. The recent FDA approval of oncolytic herpes virus T-VEC (Talimogene laherparepvec) for the treatment of metastatic melanoma has confirmed the possibility of using viruses in the clinic. As such, a promising novel treatment approach for brain tumor therapy is YB-1 targeted virotherapy. YB-1 is known to play an important role in the adenovirus life cycle, where post-adenovirus infection, YB-1 can translocate to the nucleus where

it regulates the expression of viral polymerase(111). This finding raised the possibility of targeting YB-1 nuclear accumulation with YB-1 dependent oncolytic adenoviruses. Ad-Delo3-RGD is a recombinant adenovirus in which the transactivation domain CR3 of the E1A protein is ablated to enable viral replication solely in YB-1 positive cancer cells. The Ad-Delo3-RGD virus induced significant cell lysis in various GBM cell lines(112). Of particular note, due to the YB-1 nuclear translocation induced by certain cytotoxic drugs, co-treatment with cisplatin and temozolomide significantly enhanced tumor cell killing *in vitro* and reduced tumor growth rate in a xenograft glioma mouse model(112). Likewise Ad-Delo3-RGD mediated substantial cytolysis in GBM patient-derived BTIC lines, with significantly reduced viral replication in non-malignant astrocyte cells(94). Although Ad-Delo3-RGD is yet to be tested in pediatric brain tumor lines/models, other oncolytic viruses have yielded promising results in pediatric high grade glioma, supporting the development of YB-1 targeting oncolytic viruses for patients with these tumors(113).

Concluding Remarks

To conclude, this review has described recent advances in our understanding of YB-1 as a master regulator of cancer biology, with a specific focus on pediatric brain tumors. On account of its multi-functional nature, YB-1 appears to facilitate the advancement of numerous malignant phenotypes in brain tumors, including proliferation, invasion and resistance to therapy and is a key player in the maintenance of brain tumor initiating cells. It is also highly expressed in pediatric brain tumors and correlates with poor survival outcome. Taken together, YB-1 clearly represents a protein worthy of further research within the pediatric oncology field. Further, with the

advent of YB-1 targeted therapeutic approaches such as YB-1-targeted virotherapy and oligonucleotide therapy, YB-1 has extensive potential for development as both a therapeutic target and novel biomarker for the management of pediatric brain tumors.

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Tables

Table 1. Experimentally confirmed transcriptional, translational and post-translational regulators of human *YBX1*/YB-1.

Level	Regulator	Effect	Reference
Transcriptional	C-MYC/P73	Stimulates transcription.	(47)
	TWIST1	Stimulates transcription.	(48)
	PCAF	Stimulates transcription (via Twist1 acetylation).	(50)
	PDCD4	Inhibits transcription (via direct Twist1 interaction).	(49)
	GATA-1/GATA-2	Stimulates transcription.	(51)
Translational	mTOR (mTOR Pathway)	Stimulates translation (influenced by proliferation rate).	(53)
	YB-1	Inhibits translation.	(40)
	PABP	Stimulates translation.	(52)
Post-Translational	AKT (PI3K/AKT Pathway)	Activation (via phosphorylation of S102 site).	(56)
	RSK (MAPK/ERK Pathway)	Activation (via phosphorylation of S102 site).	(55)
	FGFR1	Unknown	Reviewed in (57)
	RBBP6	Degradation (via ubiquitination-driven proteasomal cleavage).	

Figure Legends

Figure 1. *YBX1* is expressed highly in paediatric brain tumors and correlates with poor prognosis. A) *YBX1* expression levels are elevated in paediatric MB, GBM, ependymoma, anaplastic astrocytoma and DIPG compared to normal brain tissue controls. Normal cerebellum (Roth dataset) n = 9; paediatric MB (Pfister dataset) n = 71; normal brain (Harris dataset) n = 44; pGBM (Paugh dataset) n = 34; paediatric ependymoma (Pfister dataset) n = 151; paediatric anaplastic astrocytoma (Paugh dataset) n = 7; paediatric DIPG (Paugh dataset) n = 27. Expression displayed as box plots showing the sample minimum (lower line), lower quartile (bottom of box), median (line within box), upper quartile (top of box) and the sample maximum (upper line). ****P < 0.0001. Significance was assessed by either Student's t-test (paediatric MB) or one-way ANOVA analyses with Tukey's multiple comparison test (paediatric GBM, ependymoma, anaplastic astrocytoma and DIPG). B) Kaplan-Meier survival analysis of paediatric MB patients with high or low *YBX1* expression revealed high *YBX1* expression correlates with poor 5 year survival. P = 0.0002; n = 543 (Cavalli dataset). C) Kaplan-Meier survival analysis of paediatric high-grade glioma (HGG) patients with high or low *YBX1* expression revealed high *YBX1* expression correlates with poor 5 year survival. P = 0.019; n = 47 (Paugh dataset). Publically available datasets accessed using R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl).

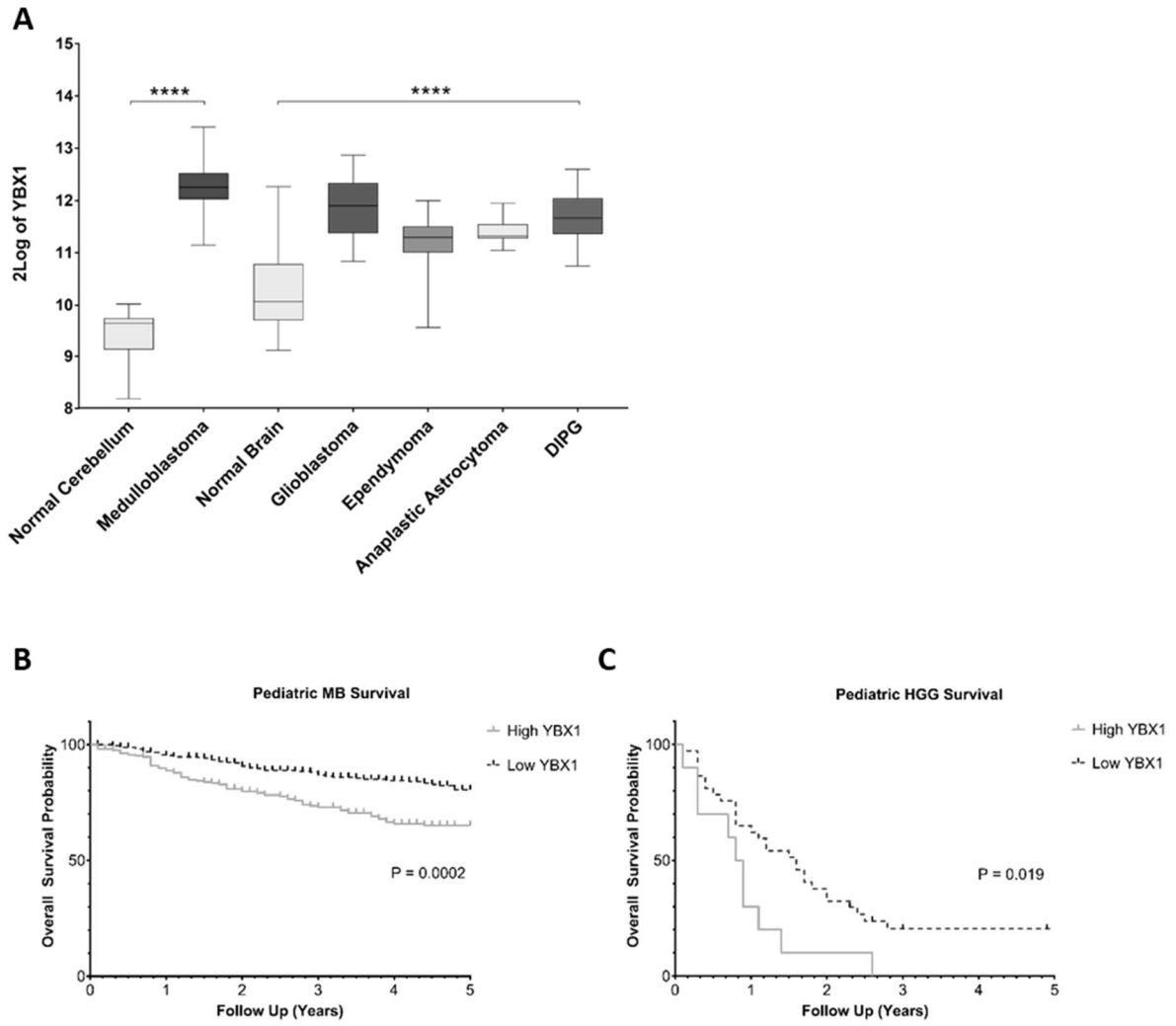
Figure 2. YB-1 protein domain organisation. YB-1 has a disordered Alanine/Proline rich (A/P) domain, a universally conserved cold shock domain (CSD) and an elongated, disordered C-terminal domain (CTD) comprising alternating clusters of positive and negatively charged amino acids. Each domain contains binding sites for various protein interactors, some of which are illustrated. Within the CTD there exists a number of proposed nuclear localisation signals (NLS) and two proposed

cytoplasmic retention signals (CRS). The CTD also contains a peptide bond linkage between Glu219 and Gly220 which is targeted by the 20S proteasome. Notable phosphorylation (orange), acetylation (green) and ubiquitination (blue) sites are also shown.

Figure 3. Proposed mechanisms of YB-1 nuclear translocation. Simplified overview of potential YB-1 nuclear transport mechanisms. YB-1 has been suggested to translocate to the nucleus upon phosphorylation by RSK/AKT or conversely is activated within the nucleus by nuclear-translocated RSK. Interaction with different proteins including splicing factor SRP30C or actin-remodelling protein WAVE3 may also promote nuclear localisation. Involvement of the 20S proteasome has also been proposed, producing either a C- or N-terminal fragment (CTF; NTF) which translocates to the nucleus. Functional P53 protein has also been suggested to promote nuclear translocation, both by direct interaction with YB-1 and by the transcriptional activation of an "effector protein" which then facilitates YB-1 nuclear transport. Dashed lines represent nuclear translocation.

Figure 4. Proposed oncogenic functions of YB-1 in brain tumors. YB-1 has been shown to facilitate the advancement of several key malignant phenotypes in brain tumors, including proliferation (A), invasion (B), resistance to therapy (C) and the maintenance of brain tumor initiating cells (BTICs; D). Oncogenic functions presented in black type have been proven experimentally in brain tumor patient samples and cell lines, whereas those in grey type have been investigated in other tumor types but are yet to be explored in brain tumors.

Figure 1


A
2Log of YBX1
15
14
13
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11
10
9
8
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Normal Cerebellum
Medulloblastoma
Normal Brain
Glioblastoma
Ependymoma
Anaplastic Astrocytoma
DIPG
B
Pediatric MB Survival
Overall Survival Probability
100
50
0
P = 0.0002
High YBX1
Low YBX1
0
1
2
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Follow Up (Years)
C
Pediatric HGG Survival
Overall Survival Probability
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50
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P = 0.019
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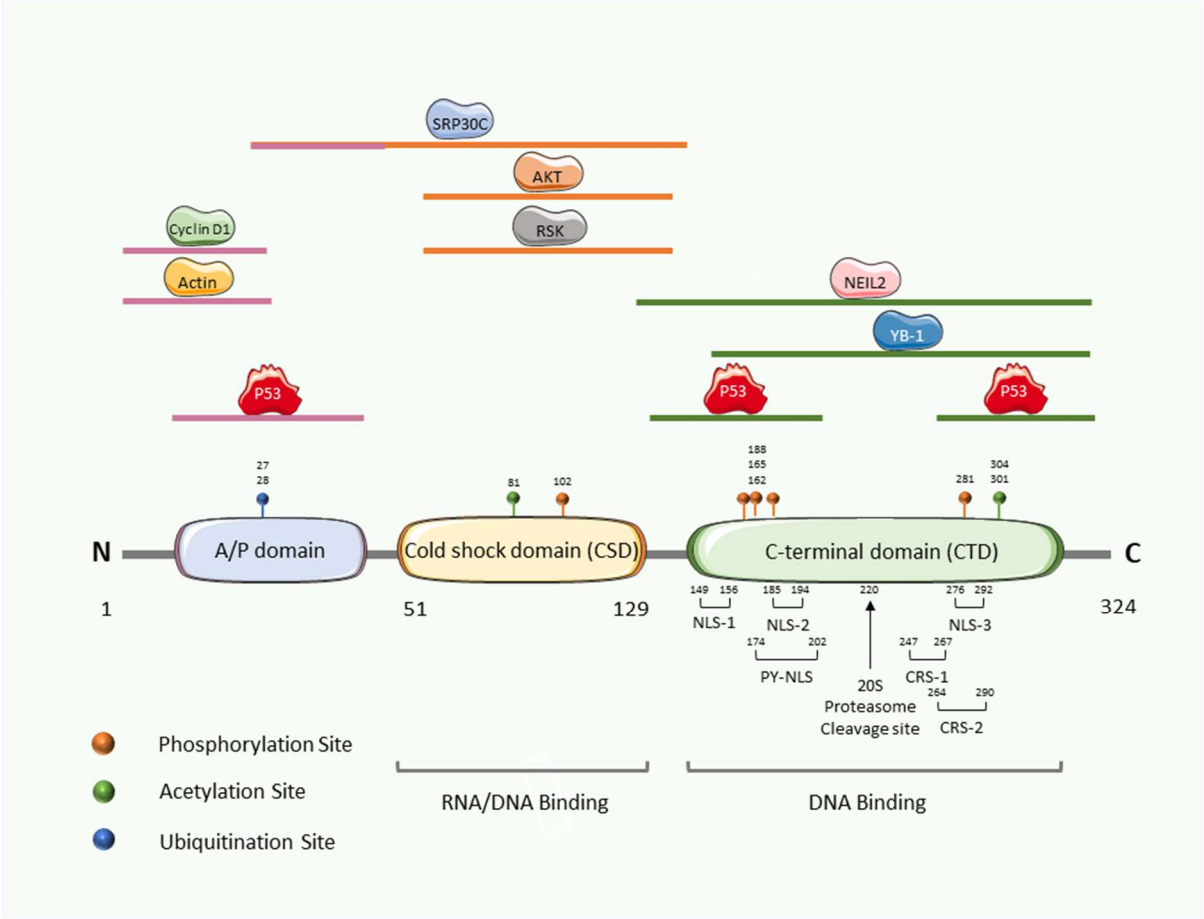
Follow Up (Years)

Figure 2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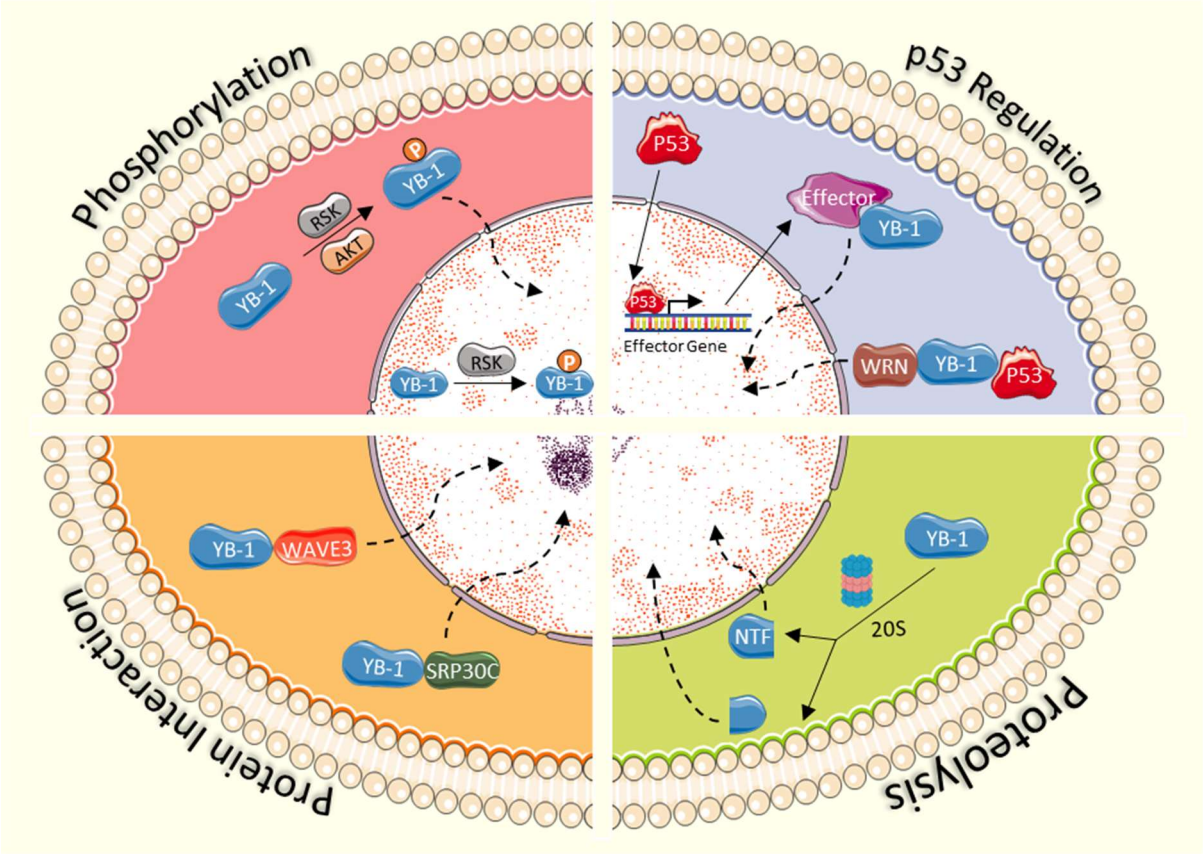
AKT
RSK
Cyclin D1
Actin
NEIL2
YB-1
P53
P53
P53
27
28
81
102
188
165
162
281
304
301
N
A/P domain
Cold shock domain (CSD)
C-terminal domain (CTD)
C
1
51
129
324
149
156
NLS-1
185
194
NLS-2
276
292
NLS-3
174
202
PY-NLS
220
20S
Proteasome
Cleavage site
247
267
CRS-1
264
290
CRS-2
Phosphorylation Site
Acetylation Site
Ubiquitination Site
RNA/DNA Binding
DNA Binding

Figure 3


Phosphorylation
YB-1
RSK
AKT
YB-1
YB-1
RSK
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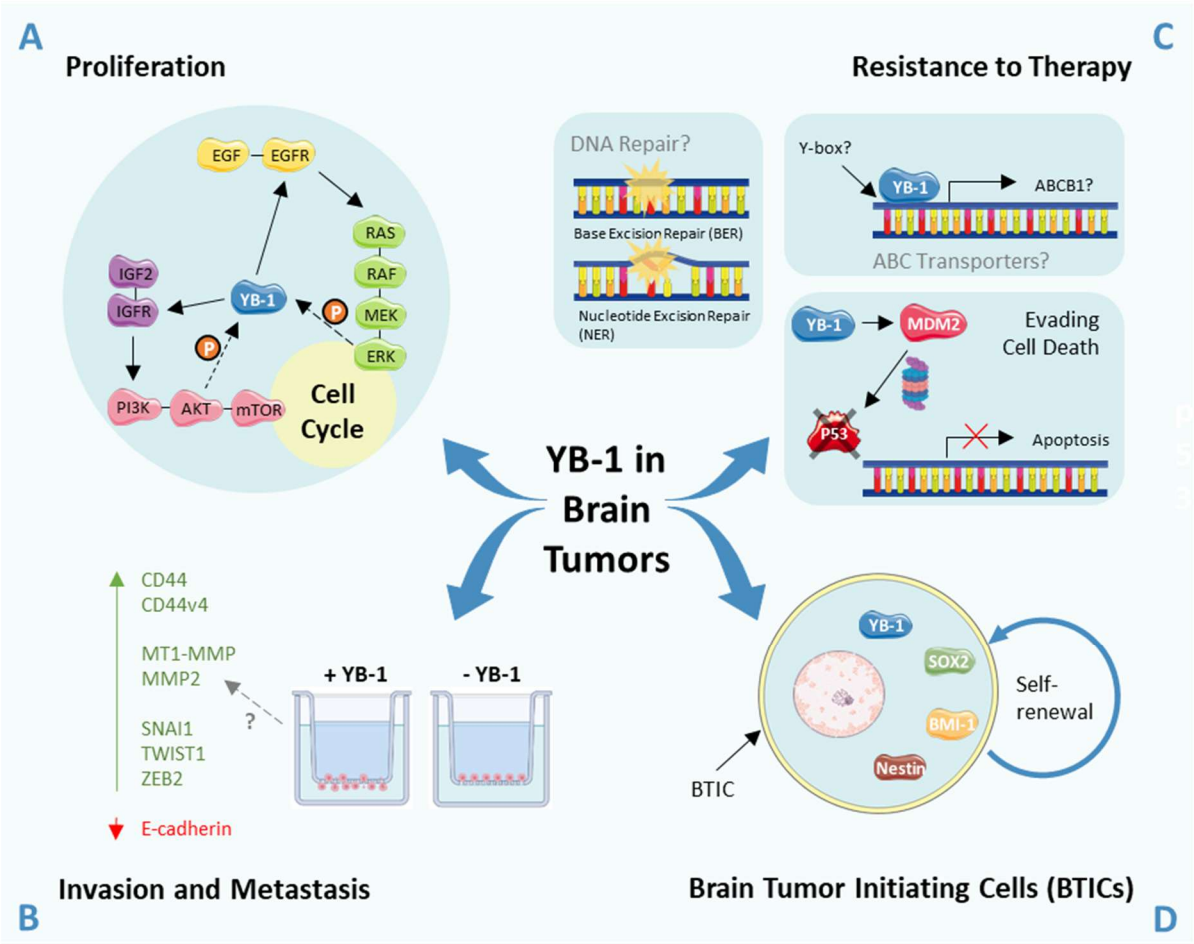
p53 Regulation
P53
Effector
YB-1
P53
Effector Gene
WRN
YB-1
P53
Protein Interaction
YB-1
WAVE3
YB-1
SRP30C
Proteolysis
YB-1
NTF
20S

Figure 4


A
Proliferation
EGF
EGFR
RAS
RAF
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ERK
IGF2
IGFR
YB-1
P
PI3K
AKT
mTOR
Cell Cycle
C
Resistance to Therapy
DNA Repair?
Base Excision Repair (BER)
Nucleotide Excision Repair (NER)
Y-box?
YB-1
ABCB1?
ABC Transporters?
YB-1
MDM2
Evading Cell Death
P53
Apoptosis
YB-1 in Brain Tumors
CD44
CD44v4
MT1-MMP
MMP2
SNAI1
TWIST1
ZEB2
E-cadherin
?
+ YB-1
- YB-1
YB-1
SOX2
BMI-1
Nestin
Self-renewal
BTIC
B
Invasion and Metastasis
Brain Tumor Initiating Cells (BTICs)
D