The Many Sides of PBRM1

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PBRM1, also known as BRG1-associated Factor 180 (BAF180) or polybromo 1 (PB1), is a polybromo protein that plays multiple roles in transcription, translation, and DNA repair. PBRM1 functions both independently and as a part of the polybromo BAF (PBAF) complex, which are part of the switching/sucrose nonfermentable (SWI/SNF) family. These complexes are large adenosine triphosphate (ATP) dependent chromatin remodeling complexes that recognize histone marks (methylation, acetylation, ubiquitination, phosphorylation, and ribosylation) to ultimately make DNA accessible for transcription.[1] There are various forms of the BAF complex depending on developmental stage and tissue type.[2] The complexes have a set of core proteins which must include one of the ATPase catalytic subunits (BRG1 or BRM). The presence of PBRM1 along with BRD7, BAF45A, and ARID2 differentiate the PBAF complex from the other BAF complexes.[3] The protein variability in BAF complexes along with their ability to recognize diverse epigenetic and nucleosome modifications leads to context dependent transcriptional control creating unique regulation of biological processes.

PBRM1 Domains

PBRM1 contains three different types of protein domains: bromodomains, bromo-associated homology domains, and a high mobility group domain (Figure 1). These distinct motifs create a protein capable of binding histones, DNA, and other proteins, making PBRM1 a key coordinator of many of the different functions of the PBAF chromatin remodeling complex.

Figure 1. Diagram of the three classes of domains present in PBRM1 and their location across the gene.
BD: bromodomain, BAH: bromo-associated homology domain, HMG: high mobility group domain.

Bromodomains

Unlike most bromodomain-containing proteins, which have one to two bromodomains, PBRM1 has six bromodomains—the most of any bromodomain-containing protein.[4] Bromodomains are acetyl-lysine binding domains and frequently interact with acetylated histones through a conserved asparagine to regulate gene expression.[5] Multiple studies have examined the histone binding patterns of the bromodomains of PBRM1 (See Table 1 for data from a few studies).[3,4,6,7] All studies indicate that PBRM1 binds to modified histones and can interact with multiple different histone acetylation marks and even methylation marks. While there is no consensus among the specific bromodomains and acetylated histone marks, there is consensus on each bromodomain having preferential binding to specific histone marks over others. Slaughter et al., was the only group to study binding of tandem bromodomains and found that the

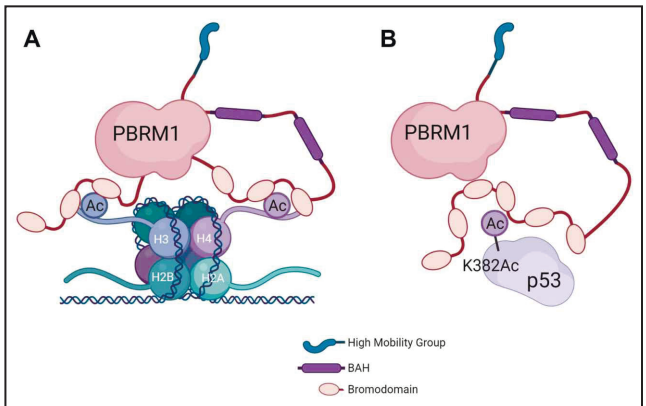

Figure 2. PBRM1 bromodomains bind acetylated histones (A) and p53 (B).

bromodomains can work cooperatively for binding at H3K14, H3K4, and/or H4K16 with bromodomain 2 and bromodomain 4 having the strongest affinity for nucleosome binding.[4] The six bromodomains of PBRM1 increase the affinity of PBAF to chromatin as compared to BAF.[8]

Bromodomain	Thompson[3]	Charlop-Powers et al[7]	Filipakopoulous et al[6]	Slaughter et al.[4]
BD1	H2AK5Ac H3K4Ac	H2BK116Ac H3K14ac	H2AK15Ac H3K56Ac H4K91Ac	
BD2	H3K9Ac	H2BK120Ac H3K115Ac H4K12Ac	H2AK36Ac H2BK85Ac H3K14Ac	H3K14Ac H3K4Me
BD3	H3K9Ac	H3K14Ac H3K115A	H2AK15	H3K4AcK14AcK18Ac
BD4	H2BK20Ac H3K23Ac	H3K36A	H3K37Ac	H3K14Ac H3K4Me
BD5		H2BK24Ac	H2BK85Ac	
BD6		H2BK116Ac	H2BK43Ac H3K56Ac H3K115Ac	

In addition to binding chromatin, the bromodomains have been shown to be key in the interaction between PBRM1 and p53. DNA damage induces acetylation on c-terminal lysine residues of p53, and since bromodomains can bind to acetyl-lysine residues it was hypothesized that the PBRM1 bromodomains would interact with p53 via this acetylation. Acetylation at lysine 382 was found to greatly enhance PBRM1-p53 binding, and bromodomain 4 was responsible for this enhanced binding. Even though bromodomain 4 is critical for the interaction at K382Ac, it is strengthened by bromodomains 2, 3, and 5. Like chromatin binding, the multiple bromodomains work collaboratively to enhance the binding to K382Ac on p53.[9]

The six bromodomains of PBRM1 overlap in their ability to bind acetylated and methylated residues on histones and p53. This leads to the question of what other acetylated marks PBRM1 might be able to "read" and what affects they have on transcription and cell growth. Such a redundancy suggests cooperation between the bromodomains and may lead to context specific differences based on developmental stages and/or tissues.

Bromo-associated homology (BAH) Domains

In addition to six bromodomains, PBRM1 contains two bromo-associated homology (BAH) domains that are highly homologous to each other.[3] While the precise functions of BAH domains in nuclear PBAF are unclear, they are thought to be important in protein-protein interactions. Thus, these domains likely play an important role in the structural integrity of the PBAF complex creating a scaffold between PBRM1 and the other components of PBFA. In addition, PBRM1 is likely to interact with other chromatin-associated proteins (i.e., transcription factors) to localize the PBRM1 complex to specific chromatin regions based on protein composition.

During interphase, PBRM1 localizes to the nucleus, however, during mitosis it has been observed at mitotic spindle poles. Interestingly, histone methyltransferase set domain containing 2 (SETD2) methylates not only histones but α-tubulin on microtubules and actin. α-TubK40me3 and ActK68me3 localize to spindle microtubules during mitosis. Karki et al., showed that PBRM1 co-localizes with the α-TubK40me3 mark from SETD2, and that, while BAH2 is stronger, both BAH domains could interact with this methylation mark. The interaction of PBRM1 with mitotic spindles recruits other members of the PBAF complex to localize here as well. These data show the important role PBRM1 and PBAF play in chromosome segregation during mitosis.[10]

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The BAH domains of PBRM1 have also been shown to be vital for completion of translesion DNA synthesis (TLS). TLS is a mechanism to replicate DNA in the presence of DNA damage and is induced by monoubiquitinated proliferating cell nuclear antigen (PCNA).11 The BAH domains of PBRM1 are required for normal levels of monoubiquitinated PCNA following UV radiation. In response to UV radiation and in the absence of PBRM1, less PCNA (both non-ubiquitinated and ubiquitinated) and Rad18, the E3 ligase that ubiquitinates PCNA, were present in the chromatin fraction. This demonstrates that PBRM1 plays a critical role in the association of these proteins to DNA during damage responses.[12] The BAH domains are the only region of PBRM1 that is required to rescue the mutant phenotype suggesting that PBRM1 may act independently of the entire PBAF complex when contributing to TLS.[13]

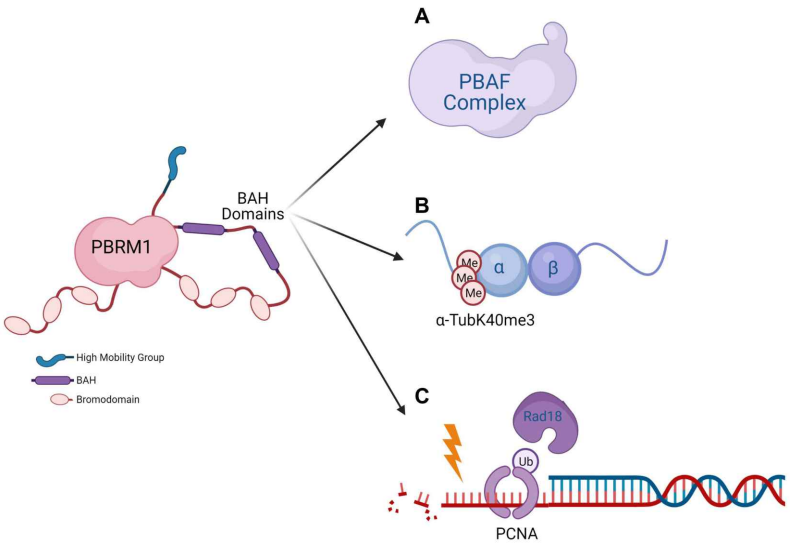

Figure 3. PBRM1 bromo-associated homology domains are critical for multiple protein-protein interactions. (A) It is hypothesized that the BAH domains are the regions of PBRM1 that interact with the other proteins in the PBAF complex. The BAH domains also interact with (B) methylated lysine 40 of alpha-tubulin which induces the recruitment of the PBAF complex to mitotic spindles during mitosis. (C) PCNA and Rad18 affecting ubiquitination of PCNA during DNA repair following UV irradiation.

The current functions of the BAH domains of PBRM1 are diverse and show that the domains can interact with a variety of proteins both in association with and without PBAF. It is unclear as to whether all BAH interactions rely on post-translational modifications or if only a subset of them do. Nonetheless, PBRM1 is known to be a key component in scenarios where large protein complexes are present or multiple protein interactions occur, and it can easily be speculated how the BAH domains might be important for any of these protein-protein interactions to help secure PBRM1 into these complex structures.

High Mobility Group (HMG) Domain

High mobility group (HMG) domains, also recognized as high mobility group boxes, bind the minor groove of DNA. This motif is frequently seen in chromosomal binding proteins and can be classified into two main groups: proteins with two HMG-box domains and a long highly acidic C-tail that bind DNA in a non-sequence specific manner and proteins that typically have one HMG-box domain and no acidic C-tail. The PBRM1 HMG domain differs from others in that it has a shortened third α-helix and an extended basic N-terminus.[14] Proteins containing HMG domains can bind to DNA in a sequence or

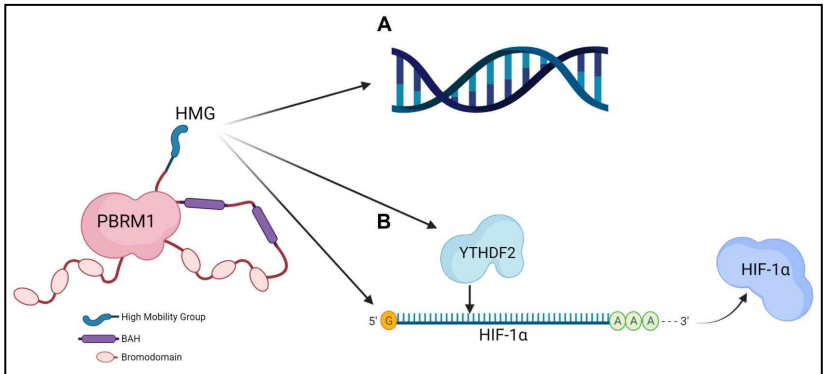

Figure 4. The high mobility group domain of PBRM1 binds to (A) DNA using a structure rather than sequence specific mechanism and (B) effects HIF-1α expression by interacting the 5'UTR and RNA binding protein YTHDF2.

structure specific mechanism. Based on sequence homology it is hypothesized that the HMG domain of PBRM1 binds DNA based on structure rather than sequence.[3]

In addition to protein-DNA interactions, the HMG domain can also help facilitate protein-protein interactions. Shmakova et al., found that PBRM1 binds to the RNA binding protein YTHDF2 via the HMG domain but not through interactions with either BAH domain. This interaction occurs in the cytoplasm independent of the PBAF complex and is critical for normal production of the protein, hypoxia-induced factor (HIF) 1α.[15]

There are three different domain families that drive the many functions of PBRM1. These domains allow PBRM1 to have distinct roles across many important cellular processes including chromatin remodeling, chromosomal segregation, DNA repair, and responses to hypoxia through its ability to bind histones, DNA, and other proteins. Because of its multifaceted nature, mutations in PBRM1 can lead to a variety of cellular disruptions prominent in cancer.

PBRM1 and Cancer

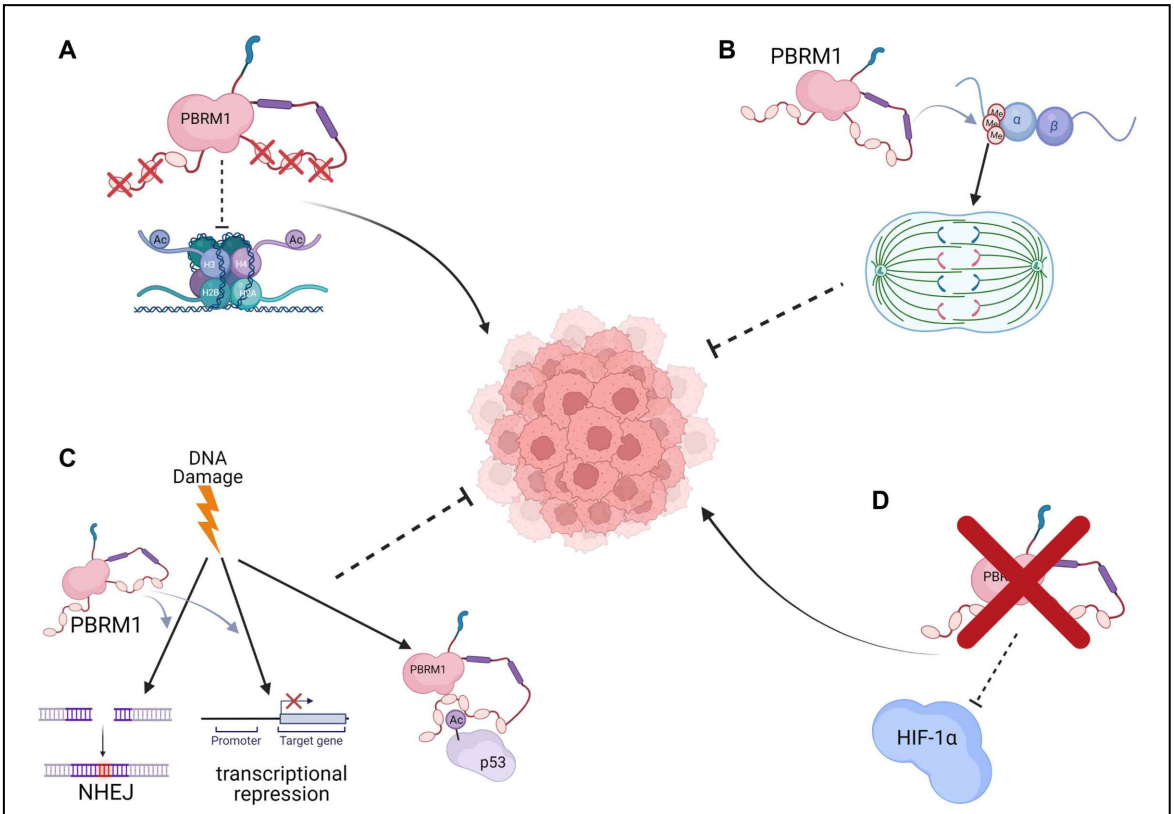

Figure 5. PBRM1 is a multifaceted protein with a variety of implications in cancer. (A) Mutations in PBRM1 bromodomains inhibits chromatin binding which can result in increased cell proliferation. (B) PBRM1s localization to mitotic spindles and role in chromosomal segregation is critical for appropriate localization of chromosomes during cellular replication which if altered can lead to the development of cancer. (C) PBRM1 plays multiple roles in appropriate induction/regulation of DNA damage responses including non-homologous end joining, transcriptional repression, and induction of p53 tumor suppressor functions. Mutations in PBRM1 can affect all of these pathways leading to improper DNA repair which can result in tumor formation. (D) PBRM1 aids in normal HIF-1α expression via translational regulation, which if altered can aid in tumor expansion due to increased availability of oxygen for consumption.

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The American Cancer Society projects that in 2022 there will be over 1.9 million new cases of cancer diagnosed and approximately 6,000 deaths from the disease.[16] The SWI/SNF complex is mutated at a rate of approximately 43%[17], which makes ATP dependent chromatin remodeling proteins some of the most frequently mutated genes in cancer.[18] PBRM1 is the second most frequently mutated gene behind ARID1A (AT-Rich Interaction Domain 1A) of the BAF/PBAF complexes[18] with a mutation frequency of almost 4% in all cancer types.[19] Around 80% of somatic mutations in PBRM1 result in loss of function.[20] PBRM1 is mutated in a variety of different cancers described using data from cBioPortal in the table below with clear cell renal cell carcinoma (ccRCC) being the most frequent.

Type of Cancer	Number of Cases	Alteration Frequency
Renal Clear Cell Carcinoma	511	32.49%
Cholangiocarcinoma	36	22.22%
Endometrial Carcinoma	586	10.92%
Esophagogastric Adenocarcinoma	514	8.56%
Melanoma	444	8.11%
Undifferentiated Stomach Adenocarcinoma	13	7.69%
Pleural Mesothelioma	87	6.9%
Bladder Urothelial Carcinoma	411	6.33%
Mature B - Cell Neoplasms	48	6.25%
Colorectal Adenocarcinoma	594	4.71%
Renal Non - Clear Cell Carcinoma	348	3.74%
Pancreatic Adenocarcinoma	184	3.26%
Head & Neck Squamous Cell Carcinoma	523	3.06%
Non - Small Cell Lung Cancer	1053	3.04%
Hepatocellular Carcinoma	369	2.98%
Cervical Squamous Cell Carcinoma	251	2.79%
Sarcoma	255	2.5%
Diffuse Glioma	513	2.73%
Thymic Epithelial Tumor	123	2.44%
Esophageal Squamous Cell Carcinoma	95	2.11%
Invasive Breast Carcinoma	1084	1.75%
Ovarian Epithelial Tumor	584	1.71%

*Data from cBioPortal for Cancer Genomics

Chromatin Remodeling

Chromatin remodelers have been shown to be highly mutated in a variety of cancers. Many of the mutations in PBRM1 are associated with the bromodomains which are critical for chromatin binding. Porter & Dykhuizen demonstrated that a mutation in any of the bromodomains outside of bromodomains 3 and 6 decreased the affinity of PBAF binding to chromatin. Additionally, they showed that a mutation in any bromodomain outside of bromodomain 3 had a moderate to dramatic effect on PBRM1's tumor suppressive phenotype.8 Similar data was observed by Slaughter et al., who found that mutations in both BD2 and BD4 were needed to diminish chromatin binding, while both the BD4 mutation and double BD2/BD4 mutations increased cell proliferation.[4] Together these data indicate that incorrectly regulated PBRM1 chromatin binding can contribute to cell proliferation and an oncogenic phenotype.

Double Strand Break Repair

Defects in DNA damage response pathways have been known to contribute to increased risk of cancer. PBRM1 plays a role in double strand DNA (dsDNA) break repair through both transcriptional repression and non-homologous end joining (NHEJ).[21] Phosphorylation at S941 (between bromodomain six and BAH) recruits PBRM1 to regions flanking dsDNA breaks leading to transcriptional silencing at these locations. This transcriptional repression is critical for rapid double strand break repair, which if delayed could lead to mutations. In addition, this localization of PBRM1 to dsDNA breaks inhibits the resection necessary for homologous recombination thereby facilitating NHEJ. Mutations in PBRM1 have been shown to have a defect in dsDNA break repair and the associated transcriptional repression.[21]

Methylated Microtubules

The cell cycle is a highly coordinated series of events that are tightly regulated. Most cells spend their time in interphase performing their standard set of functions. However, when cells transition out of interphase and toward replication, they move into S phase to duplicate their DNA. These cells then proceed through mitosis to ensure proper segregation of each chromosome into daughter cells. During interphase, PBRM1 is located in the nucleus, but during mitosis, PBRM1 is found mainly at the poles of the mitotic spindle. PBRM colocalizes with SETD2 (histone methyltransferase set domain containing 2) methylated α-tubulin (α-TubK40me3). Karki et al., showed that PBRM1 interacts with α-tubulin through its BAH domains via methylations rather than its bromodomains via acetylation as previously shown for chromatin binding. The interaction of PBMR1 with α-tubulin recruits additional members of PBAF to microtubules, which is required for proper chromosomal segregation. Two PBRM1 oncogenic mutations found in BAH domains reduced binding of PBRM1 to α-tubulin while leaving chromatin binding and PBAF formation at normal levels. This shows that mutations in the BAH domains that reduce α-tubulin binding result in genomic instability even though the other PBRM1 functions remain intact. [10]

p53

p53 is a well-known tumor suppressor whose function relies heavily on its transcriptional activity. This activity is regulated by the acetylation of lysine residues which can be induced by multiple stress signals including DNA damage.[22] As discussed earlier, bromodomains are acetyl-lysine binding domains, and so it was hypothesized that the bromodomains of PBRM1 might interact with p53. Cai et al., found that bromodomain four binds to K382Ac and this interaction with p53 increases upon DNA damage. Mutations in bromodomain four disrupt PBRM1-p53 interactions resulting in decreased tumor suppressor functions including expression of p21. This reduction in p21 expression increased the amount of cells progressing to S phase after DNA damage.[9] The PBRM1-p53 interaction is critical for cell cycle regulation after cellular stress and is one of the mechanisms that mutations in PBRM1 lead to cancer.

HIF-1

Hypoxia, low oxygen levels, is frequently observed in solid tumors where high proliferation rates result in dense masses of cells limiting the oxygen supply to the center of the tumor. Given that oxygen homeostasis is critical for the energy maintenance of cells, tumor cells adapt to these low oxygen conditions by inducing expression of genes involved in angiogenesis, glucose metabolism, and cell migration. HIF-1 is a transcription factor frequently activated during hypoxia. HIF-1 mediates the induction of glycolytic metabolic pathways, angiogenesis factors like vascular endothelial growth factor (VEGF), and tumor metastasis. HIF-1 is a heterodimeric transcription factor composed of HIF-1α and HIF1β. HIF-1β is constitutively expressed, but HIF-1α is expressed under hypoxic conditions.23 During standard oxygen conditions, HIF-1α is targeted for proteasomal degradation by sequential proline hydroxylation (by proline 15hydroxylases) and polyubiquitination (by Von Hippel-Lindau (VHL) E3 ubiquitin ligase).

VHL and PBRM1 are the two most commonly mutated genes in clear cell renal cell carcinoma (ccRCC), that both play 19a role in HIF-1 α expression. Depletion of PBRM1 decreased expression of HIF-1α and HIF-1α controlled genes but had no effect on HIF-1α protein stability or mRNA levels indicating PBRM1 functions at the translational level. PBRM1 can bind to HIF-1α mRNA via the 5' UTR. Also as discussed previously, the HMG domain of PBRM1 binds to YTHDF2, a m6A methylation reader. HIF-1α has been shown to be methylated by m6A, which can be bound by YTHDF2, but this interaction is dependent on PBRM1. These interaction between HIF-1α mRNA, YTHDF2, and PBRM1 are critical for proper 15translation and expression of HIF-1 α.

Immune Checkpoint Inhibitors

Over the last decade immune checkpoint inhibitor treatments have increased in popularity as a mechanism to increase cancer patient survival. The tumor environment is complex and highly variable with degrees of immune cell infiltration. Tumor cells adapt to evade the immune system often by developing signals to suppress the host's immune response, which can occur by activating immune checkpoint pathways. Therefore, targeting these suppressive pathways can expose the tumor to immune responses that can decrease tumor size and burden. However, not all cancers or patients are alike and so immune checkpoint inhibitor treatments do not have the same 24 successful outcome in all patients. Much work is currently being performed to determine characteristics that will differentiate between patients who will have success with immune checkpoint inhibitor therapy vs those who will not. Due to the high mutation rate of PBRM1 in cancer, it was examined as a possible biomarker for differentiating outcomes of immune checkpoint inhibitor therapy. Many studies have been performed to examine PBRM1 mutations 25–29and immune therapy responses. However, the studies provide conflicting data on whether presence of PBRM1 is beneficial or detrimental for immune checkpoint therapy.

Early studies suggested that that tumors with a loss of function mutation in PBRM1 may be more sensitive to immune checkpoint inhibitors.[26,27] A study by Miao et al., found that loss of PBRM1 increased responsiveness to immune checkpoint inhibitor treatment (anit-PD-1 and anti-CTLA-4) in ccRCC. This study compared genomic sequencing of tumors from metastatic ccRCC patients who had clinical benefits from anti-PD-1 treatment to those who did not.[27] Along these lines, Pan et al., found, using RNA-sequencing data and data from Tumor Immune Estimation Resource, that PBRM1 and ARID2 expression levels were negatively correlated with expression of cytotoxic T cell effector genes. The researchers also used a mouse melanoma model to examine mechanisms that determine resistance to immune therapies and found that disrupting PBRM1 function in cells resistant to anti-PD-1 and anti-CTLA-4 treatment rendered them sensitive to these treatments.[26]

Conversely, additional studies established that loss of PBRM1 function increased resistance to immune checkpoint inhibitor treatments.[19,28] Yang et al. used data from cBioPortal and Tumor Immune Estimation Resource to examine overall patient survival, tumor mutational burden, and immune infiltrates. They found that mutations in PBRM1 typically led to a decreased expression of PBRM1 in almost all the cancers examined as compared to normal tissues except for stomach adenocarcinoma and kidney chromophobe.19 Cancer patients with PBRM1 mutations had decreased overall survival (poor prognosis) as compared to patients without PBRM1 mutations when receiving anti-PD-1 or PD-L1 treatments.[19] Additionally, Liu et al., found that loss of PBRM1 decreased the expression of interferon-γ target genes, which play an important part in T cell infiltration. This correlated with the observation of lower levels of immune cells in the tumor environments where PBRM1 loss of function mutations were present. The study also found in analyses of PBRM1 knockout mouse tumors, pre-malignant mouse kidneys, and patient cohorts had increased resistance to immune checkpoint inhibitor therapy.[28]

To further investigate correlations between PBRM1 and immune checkpoint inhibitors, expression of PBRM1, PD-L1, and CD8 were examined in a variety of tissue samples. The summary table below shows the breakdown of PBRM1, PD-L1, and CD8 expression levels in multiple cancer and normal tissues.

Approximately 20% of the of the cancer samples examined expressed PD-L1 and these were split between 40% having low PBRM1 levels and 60% having high PBRM1 levels. Figure 6 provides an example of two different breast cancer samples that differed in PBRM1 expression levels, while still expressing PD-L1 and CD8. The PBRM1 data was collected using an antibody to the n-terminus of the protein, which could detect truncated proteins. The selection of antibodies to different regions of PBRM1 may be key in differentiating between full length and truncated proteins. This information could shed light on some of the discrepancies between the data on the role of PBRM1 and checkpoint inhibitor therapy.

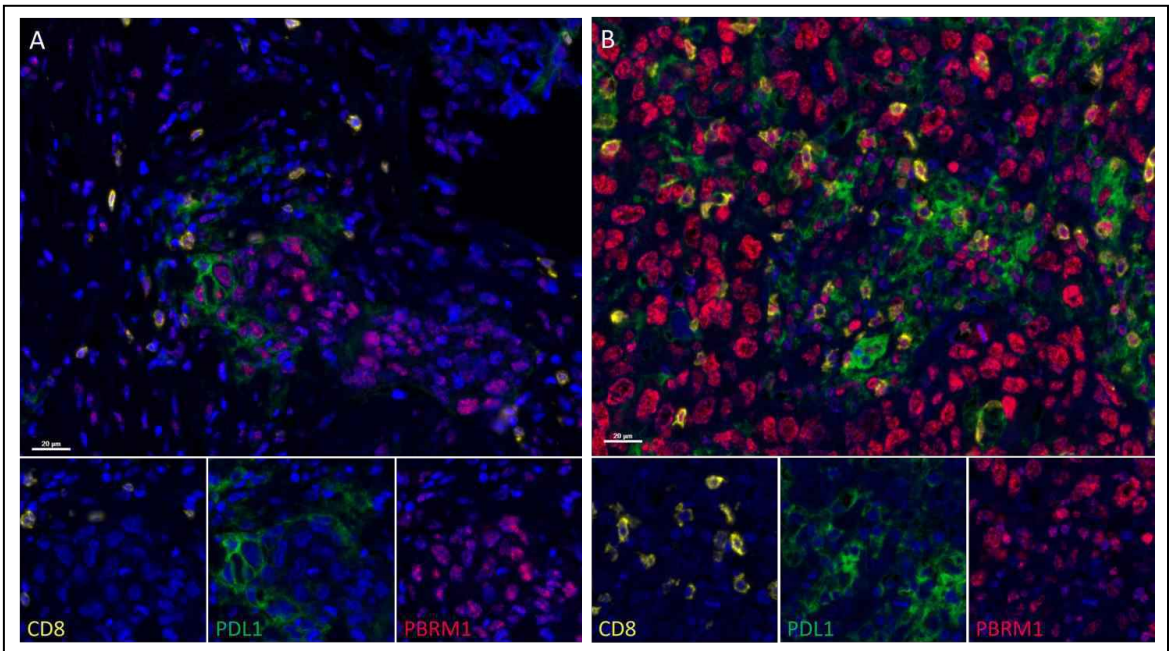

Figure 6. PBRM1 (red), PD-L1 (green), and CD8 (yellow) expression levels in two different breast cancer samples by mIF. Rabbit anti-PBRM1 recombinant monoclonal [BL-39-2C3] (A700-019), rabbit anti-PD-L1 monoclonal [BLR020E] (A700-020), and rabbit anti-CD8 monoclonal [BLR044F] (A700-044). Secondary: HRP-conjugated goat anti-rabbit IgG (A120-501P). Substrate: Opal™ 520, 620, 690. Counterstain: DAPI (blue).

Together these studies reveal conflicting data regarding the effect of PBRM1 mutations on checkpoint inhibitor treatments indicating more work still needs to be done to understand differences between cancer types and PBRM1 mutations.

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Tissue Sample	# Samples	PBRM1			PD-L1		CD8		
		None	Low	High	No	Yes	None	Low	High
Burkitt Lymphoma	1		1			1		1	
Lymph Node Cancerous	3	1	1	1	3			2	1
Lymph Node (Normal)	2		2		2			1	1
Hodgkin's Lymphoma NECK	1			1	1				1
Kidney Non-Hodgkin's B-Cell Lymphoma	1			1	1				1
Renal Cell Carcinoma	1	1			1			1	
Kidney (Normal)	1	1			1			1	
Osteosarcoma	1		1		1			1	
Gastric Cancer	1			1	1			1	
Linitis plastic	1		1		1		1		
Colon Cancer	4	1	1	2	4			2	2
Colon (Normal)	1		1		1			1	
Melanoma	2		2		2				2
Skin Squamous Cell Carcinoma	3			3	2	1		1	2
Head and Neck Squamous Cell Carcinoma	1			1		1			1
Seminoma	2		2		2			1	1
Prostate Hyperplasia	3	1	2		3				3
Prostate Cancer	3		1	2	3				3
Bladder Cancer	1			1	1			1	
Ovarian Cancer	5		1	4	3	2		4	1
Breast Cancer	8		3	5	6	2	1	4	3
Lung Cancer	4			4	3	1			4
Lung (Normal)	1		1		1			1	
Liver Cancer	2		2			2			2
Liver (Normal)	1		1			1			1
Glioblastoma	1			1	1		1		
Astrocytoma	1		1		1			1	
Cerebellum (Normal)	1			1	1			1	
Tonsil (Normal)	5		1	4		5	1	1	3
Appendix (Normal)	1			1	1				1
Inflamed Appendix			1			1			1
Spleen (Normal)	3	2	1		1	2			3
Skeletal Muscle (Normal)	1	1			1			1	
Placenta (Normal)	1		1			1		1	

Conclusions

PBRM1 is a critical component of the PBAF complex traditionally known to play an important role in chromatin remodeling. This review has shown the many different functions of PBRM1 associated with and independent of PBAF. This large multidomain protein facilitates protein-protein, protein-DNA, and protein-RNA interactions. It is frequently mutated in cancers resulting in dysregulated chromosomal segregation, DNA repair, hypoxic regulation, and transcription. Even with all these functions of PBRM1 identified, more work needs to be done to elucidate specifics of PBRM1 in different cancer types and whether it can be utilized as a biomarker for cancer and immune checkpoint inhibitor therapy.

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