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Regulation of the phosphoprotein phosphatase 2A system and its modulation during oxidative stress: A potential therapeutic target?

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ABSTRACT

Phosphoprotein phosphatases are of growing interest in the pathophysiology of many diseases and are often the neglected partner of protein kinases. One family member, PP2A, accounts for dephosphorylation of ~55-70% of all serine/threonine phosphosites. Interestingly, dysregulation of kinase signalling is a hallmark of many diseases in which an increase in oxidative stress is also noted. With this in mind, we assess the evidence to support oxidative stress-mediated regulation of the PP2A system

In this article, we first present an overview of the PP2A system before providing an analysis of the regulation of PP2A by endogenous inhibitors, post translational modification, and miRNA. Next, a detailed critique of data implicating reactive oxygen species, ischaemia, ischaemia-reperfusion, and hypoxia in regulating the PP2A holoenzyme and associated regulators is presented. Finally, the pharmacological targeting of PP2A, its endogenous inhibitors, and enzymes responsible for its post-translational modification are covered.

There is extensive evidence that oxidative stress modulates multiple components of the PP2A system, however, most of the data pertains to the catalytic subunit of PP2A. Irrespective of the underlying aetiology, free radical-mediated attenuation of PP2A activity is an emerging theme. However, in many instances, a dichotomy exists, which requires clarification and mechanistic insight. Nevertheless, this raises the possibility that pharmacological activation of PP2A, either through small molecule activators of PP2A or CIP2A/SET antagonists may be beneficial in modulating the cellular response to oxidative stress. A better understanding of which, will have wide ranging implications for cancer, heart disease and inflammatory conditions.

Contents

Abbreviations: Akt, protein kinase B; CAMK IV, calcium calmodulin kinase IV; CDC14s, protein tyrosine phosphatase CDC14; CDK1, cyclin dependent kinase 1; CIP2A, cancerous inhibitor of PP2A; CREB, cAMP response element binding protein; DUSP, dual specificity phosphatase; eNOS, endothelial nitric oxide; ENSA, α-endosulfine; ERK, extracellular signal related kinase; FAM122A, family with sequence similarity 122A; FCP-1, TFIIF-associating component of RNA polymerase II CTD phosphatase; GSH, glutathione; GSK3β, glycogen synthase kinase 3β; GWK, Greatwall kinase; HDAC, histone deacetylase; HIF1α, hypoxia inducible factor 1α; HEAT, Huntingtin-elongation-A subunit-TOR; I1PP2A, Inhibitor 1 of PP2A; ICAM, intercellular adhesion molecule; I/R, ischaemia reperfusion; JNK, Jun N-terminal kinase; LCMT-1, Leucine carboxyl methyltransferase 1; MAPK, mitogen activated protein kinase; MCAO, middle cerebral artery occlusion; miRNA, micro ribonucleic acid; NADPH, reduced nicotinamide adenine dinucleotide phosphate; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; NOX, NADPH oxidase; OA, okadaic acid; OGD, oxygen glucose deprivation; PKA, Protein kinase A; PME-1, Phosphatase methylesterase 1; PP2A, Protein phosphatase 2A; PTENs, phosphatase and tensin homologs; PTPA, serine/threonine-protein phosphatase 2A activator; ROS, reactive oxygen species; SMAP, small molecule activator of PP2A; SOD, superoxide dismutase; Src, Proto-oncogene tyrosine-protein kinase; SV40ST, Soluble monomeric simian virus 40 (SV40) small-t antigen..

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1. Introduction

Coordinated protein phosphorylation, an essential mechanism regulating cell function and signalling, is mediated through the opposing action of kinases and phosphatases. Approximately 30% of all proteins undergo kinase mediated phosphorylation (Hunter, 2000) at serine, threonine and tyrosine residues (Barford, Das, & Egloff, 1998; Gallego & Virshup, 2005; Johnson & Lewis, 2001), with serine making up >85% of all phosphorylated residues (Olsen et al., 2006). To counterbalance the action of the 500 or so protein kinases encoded in the human genome (Manning, Whyte, Martinez, Hunter, & Sudarsanam, 2002), there are approximately 200 genes encoding protein phosphatases (Cohen, 2001). The protein phosphatases are highly conserved across species, and in eukaryotic cells control processes as diverse as cell division, differentiation, apoptosis (Bononi et al., 2011), secretion, gene regulation and cellular metabolism (Hunter, 1995). Despite the obvious importance of protein phosphatases, less is known about them compared to the protein kinases.

Protein phosphatases are classified based on substrate specificity and catalytic mechanism. Within the superfamily, there are two major families and several subfamilies with multiple members (Fig. 1). These are the protein serine/threonine phosphatases, and protein tyrosine phosphatases (Moorhead, Trinkle-Mulcahy, & Ulke-Lemee, 2007; Shi, 2009b). The protein serine/threonine phosphatases are sub-classified into three structurally distinct families: phosphoprotein phosphatases, metal-dependent protein phosphatases and aspartate-based phosphatases. The protein tyrosine phosphatase family is divided into 3 main families (Class I-III) on the basis of structure and gene sequence (Moorhead et al., 2007) with several sub-families. Regarding the protein serine/threonine phosphatases, protein phosphatase 2A (PP2A) is highly conserved and ubiquitously expressed in eukaryotic cells (Cohen, Brewis, Hughes, & Mann, 1990). Importantly, PP2A and PP1, another serine/threonine phosphatase, account for ~90% of all cellular phosphatase activity in the heart (Heijman, Dewenter, El-Armouche, & Dobrev, 2013). As such, the catalytic activity of PP2A is tightly regulated. Functionally, PP2A plays a key role in numerous cell signalling pathways including those that regulate cardiac contraction, the cell cycle, cellular metabolism, cell migration, and cell survival (Lubbers & Mohler, 2016; Seshacharyulu, Pandey, Datta, & Batra, 2013; Wlodarchak & Xing, 2016). From a clinical perspective, it is emerging as an important therapeutic target (Baskaran & Velmurugan, 2018). For the present review, we give an overview of the structure and regulation of PP2A, before proceeding to discuss its modulation by oxidative stress, and emerging pharmacology.

2. The PP2A holoenzyme

2.1. Structural organisation

PP2A primarily exists as a trimeric holoenzyme consisting of a catalytic subunit (PP2Ac) and scaffolding subunit (PP2Aa/PR65) in combination with several regulatory subunits (Price & Mumby, 2000; Wijnker et al., 2011). However, approximately 30% of PP2A complexes

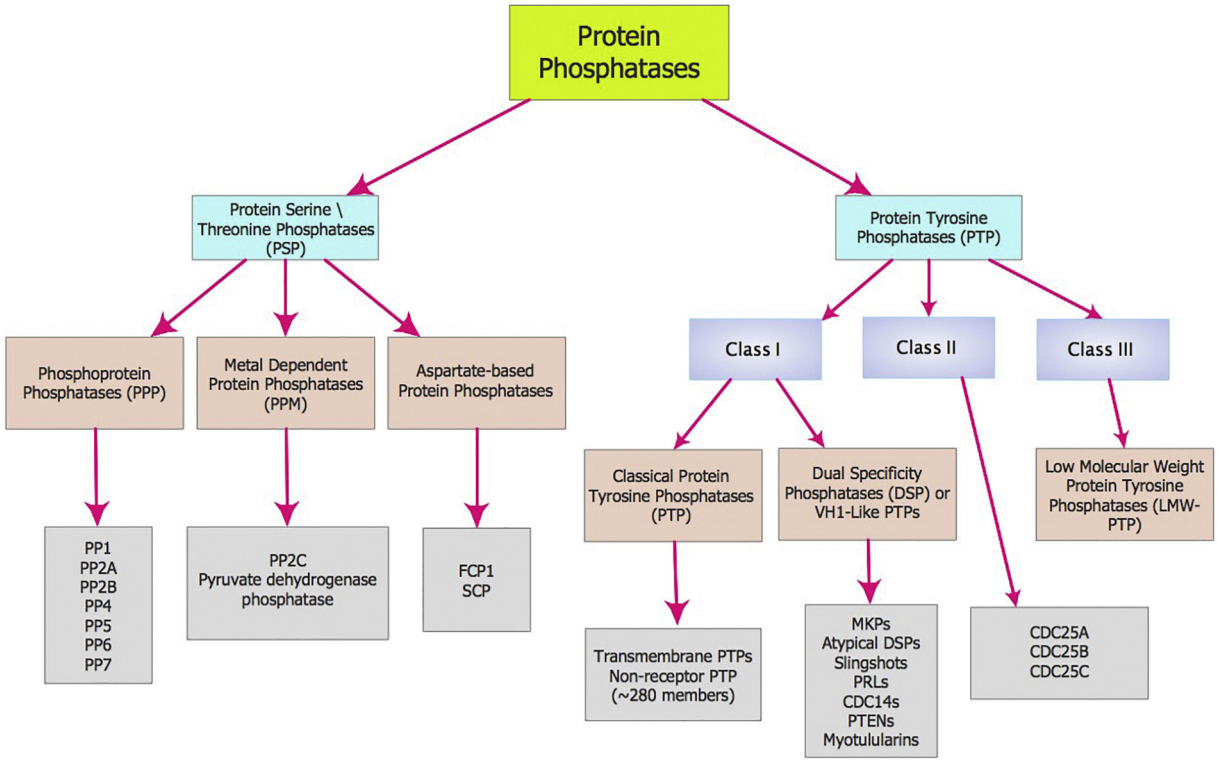

Fig. 1. Classification of the protein phosphatases. Abbreviations: CDC14s, protein tyrosine phosphatase CDC14; FCP-1, TFIIF-associating component of RNA polymerase II CTD phosphatase; MKPs, mitogen-activated protein kinase phosphatases; PRLs, phosphatases of regenerating liver; PTENs, phosphatase and tensin homologs; SCP, small CTD phosphatase.

exist as a 'core dimer' composed of a catalytic and scaffolding subunit (PP2A-CB) (Kremmer, Ohst, Kiefer, Brewis, & Walter, 1997).

There are two isoforms of the catalytic subunit (PP2Acα and PP2Acβ; Fig. 2, Table 1), which share 97% sequence homology, the most prevalent isoform being PP2Acα (Khew-Goodall & Hemmings, 1988; Stone, Hofsteenge, & Hemmings, 1987). Importantly, both share a unique C-terminal tail containing a TPDYFL motif (Cho & Xu, 2007; Xing et al., 2006), that is spatially located between the scaffolding and regulatory subunits in the holoenzyme (Cho & Xu, 2007; Xing et al., 2006). This motif plays a fundamental role in regulating catalytic activity and holoenzyme assembly (Ogris, Gibson, & Pallas, 1997; Wei et al., 2001). Interestingly, there is a variant of PP2Acα that is catalytically inactive and exhibits augmented binding to α4 protein (PP2A inhibitor) (Murata, Wu, & Brautigan, 1997), but does not bind to the scaffolding subunits (Migueleti, Smetana, Nunes, Kobarg, & Zanchin, 2012).

The scaffolding subunit also has two isoforms (PR65α and PR65β) (Cho & Xu, 2007; Eichhorn, Creyghton, & Bernards, 2009) which share 87% sequence homology. Structurally, they consists of 15 HEAT (huntingtin-elongation-A subunit-TOR) repeats, which are short pairs of interacting helices linked by a tight 1–3 residue turn that forms a horseshoe shape (Hemmings et al., 1990). It is through the HEAT repeats that the catalytic and B56 regulatory subunits bind to the scaffolding subunit to form the trimeric holoenzyme. The catalytic subunit binds to HEAT repeats 11-15 through a mix of hydrogen, hydrophobic and ionic bonding (Cho & Xu, 2007; Xing et al., 2006). This not only keeps the active site of the catalytic subunit exposed and accessible to substrate, but facilitates binding of the carboxyl terminus of PP2Acα to a surface groove at the interface between the scaffolding and a regulatory subunit enabling recruitment of the latter (Cho & Xu, 2007).

The PP2A regulatory subunits are divided into 4 subfamilies, B, B′, B″ and B‴ (Fig. 2) which share little sequence similarity (Xu et al., 2006). The B or PR55 subfamily (Table 1) has 4 members encoded by PPP2R2A/PR55α, PPP2R2B/PR55β, PPP2R2C/PR55γ, and PPP2R2D/PR55δ (Janssens & Goris, 2001). PP2R2A and PP2R2D are widely expressed, while PP2R2B and PP2R2C are principally expressed in the brain (Strack, Zaucha, Ebner, Colbran, & Wadzinski, 1998). The B′ or B56 subfamily, is the largest subfamily with 5 members, encoded by PPP2R5A/B56α, PPP2R5B/ B56β, PPP2R5C/B56γ, PPP2R5D/B56δ, and PPP2R5E/B56ε (Table 1). Although PPP2R5A and PPP2R5C are widely expressed, they are primarily found in heart and skeletal muscle, while PPP2R5B and PPP2R5D are principally expressed in brain (Csortos, Zolnierowicz, Bako, Durbin, & Depaoli-Roach, 1996; Mccright & Virshup, 1995; Tehrani, Mumby, & Kamibayashi, 1996). The third subfamily, B″ or PR72 family, has three members, encoded by PPP2R3A, PPP2R3B, and PPP2R3C (Degrande et al., 2013), and several isoforms (Janssens & Goris, 2001). PPP2R4 is the only member of the B‴ family and is often referred to as PR53 (Janssens et al., 2000) or serine/threonine-protein phosphatase 2A activator (PTPA). This is because it upregulates the intrinsic phosphotyrosyl phosphatase activity of PP2Ac upon association with the PP2A core dimer (Zolnierowicz, 2000).

Table 1
Details of the PP2A subunit families, subfamilies, gene names, isoforms, and alternative names.

PP2A subunit	Family	Gene Name	Isoform	Alternative Name
Catalytic subunits "C"	C	PPP2CA	PP2A-cα	
		PPP2CB	PP2A-cβ	
Scaffolding subunits "A"	A	PPP2R1A	PP2A-Aα	PR65α
		PPP2R1B	PP2A-Aβ	PR65β
Regulatory subunits "B"	B family (B55/ PR55/ PPP2R2)	PPP2R2A	PP2A-Bα	B55α
		PPP2R2B	PP2A-Bβ	B55β
		PPP2R2C	PP2A-Bγ	B55γ
		PPP2R2D	PP2A-Bδ	B55δ
	B' family (B56/ PR61/PPP2R5)	PPP2R5A	PP2A-B'α	B56α
		PPP2R5B	PP2A-B'β	B56β
		PPP2R5C	PP2A-B'γ1, γ2, γ3	B56γ
		PPP2R5D	PP2A-B'δ1, δ2, δ3	B56δ
		PPP2R5E	PP2A-B'ε	B56ε
	B″ family (PR72 /PPP2R3)	PPP2R3A	PP2A-B″α1, α2	PR72/PR130
		PPP2R3B	PP2A-B″β	PR48
		PPP2R3C	PP2A-B″γ	PR59, G5PR
	B‴ family (PR93/ PR110)	PPP2R4	PP2A-B‴	PTPA/PR53

Binding of the regulatory subunits to the catalytic and scaffolding subunits to form the holoenzyme differs by class. For example, the B subunits bind to HEAT repeats in the scaffolding subunit, but have little interaction with the catalytic subunits when forming the holoenzyme (Xu et al., 2006; Xu, Chen, Zhang, Jeffrey, & Shi, 2008). In contrast, the B' subunits interact with both the scaffolding subunit (HEAT repeat 2-8) and catalytic subunit (Shi, 2009a) to form a compact and rigid holoenzyme (Martin, Kettmann, & Dequiedt, 2010). The B″ subunits differ yet again as they bind to the scaffolding subunit through two calcium binding EF hands (EF1 and EF2) (Janssens et al., 2003). It is the multiplicity of catalytic, scaffolding, and regulatory subunits which culminate in the formation of 96 unique holoenzyme complexes (Janssens, Longin, & Goris, 2008). This at least in part explains how one 'enzyme' can show substrate specificity and regulate diverse physiological and pathophysiological processes.

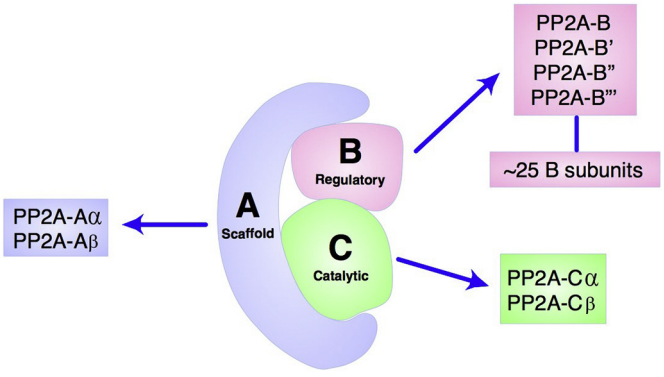

Fig. 2. Structural organisation of the PP2A holoenzyme showing the catalytic (C), regulatory (B) and scaffolding subunits (A) and major family members

2.2. Determination of PP2A substrate specificity and localisation

Work from several groups highlights the importance of the regulatory subunits in determining substrate specificity and subcellular localisation of PP2A. For example, inclusion of the B55α (PPP2R2A) subunit in the PP2A holoenzyme is required for PP2A-mediated dephosphorylation of p107 in rat chondrocytes (Kolupaeva, Daempfling, & Basilico, 2013) and in human osteosarcoma and glioblastoma cells (Jayadeva et al., 2010). The B55α subunit also targets PP2A towards phospho-β-catenin (Zhang et al., 2009) and an AP-1 complex in tumour cells (Gilan et al., 2015). It is worth noting that heat shock transcription factor 2 (HSF2) can modulate PP2A activity by preventing assembly of the holoenzyme via steric hindrance, as it binds to the same binding site on PP2Ac as B55 (Hong & Sarge, 1999). In contrast, B56γ determines PP2A substrate specificity during cardiac development in mice (Varadkar et al., 2014), while B56α modulates organogenesis through altered Wnt/β-catenin signalling (Martens et al., 2004; Seeling et al., 1999). PP2A-B56 also mediates insulin signalling and lipid metabolism via dephosphorylation of Akt in *C. elegans* and Drosophila (Padmanabhan et al., 2009; Vereshchagina, Ramel, Bitoun, & Wilson, 2008). Finally, in cortical and hippocampal neurones, PR72 (B″ family member) is involved in PP2A-mediated dephosphorylation of dopamine and cAMP-regulated phosphoprotein (DARPP-32) (Ahn, Sung, et al., 2007).

The association of PP2A with intracellular structures and localisation to cellular compartments is also dependent upon the B subunit composition of PP2A. In rat brain, B55 (α, β) localises PP2A to the cytosolic and nuclear compartments, whereas the γ isoform directs it towards unidentified cytoskeletal structures (Strack et al., 1998). Similarly, B56 (α, β, and ε) localises PP2A in the cytoplasmic compartment (Mccright, Rivers, Audlin, & Virshup, 1996), whereas the δ and γ isoforms guide it towards the nucleus (Mccright et al., 1996) or sub-nuclear structures associated with transcription factor assembly (Gigena, Ito, Nojima, & Rogers, 2005). It should be noted that B56δ moves between the nucleus and cytosol in a cell cycle stage dependent manner (Tehrani et al., 1996), indicating that localisation can be dynamic. Given the importance of the regulatory subunits in determining substrate specificity and localisation of PP2A, in conjunction with the diversity of subfamily members, it is an area justifying more in-depth investigation.

3. Regulation of PP2Ac and its holoenzyme

3.1. Transcriptional regulation of PP2Ac

PP2Ac is encoded by two separate genes, PPP2CA and PPP2CB, which are expressed in a tissue specific manner. Importantly, expression of PP2Acα mRNA is ~ 10-fold higher than that of PP2Acβ in mammals (Khew-Goodall & Hemmings, 1988). Initial analysis of the promoter region of PPP2CA and PPP2CB show that they lack TATA and CCAAT motifs but contain several GC rich regions. Vis-à-vis PP2Acα, its promoter is postulated to contain several Sp1 (stable protein 1) and a CRE (cAMP response element) binding motif (Khew-Goodall, Mayer, Maurer, Stone, & Hemmings, 1991). This has been confirmed experimentally using serially truncated PP2Ac promoter luciferase constructs to show that CRE and Sp1 binding motifs localised in a region 240 bp upstream of the transcription initiation start site are key in the transcriptional regulation of PPP2Acα (Sunahori, Juang, & Tsokos, 2009). Additionally, forskolin, caffeine, thapsigargin (via calcium release) and hepatitis C all increase PP2Aα promoter activity in a CREB (cAMP response element binding protein) dependent manner (Christen, Treves, Duong, & Heim, 2007; Kitagawa, Shima, Sasaki, & Nagao, 1991; Liu, Chen, Cheng, Lin, & Chang, 2013; Yasuoka et al., 2004). The latter is exploited by the hepatitis C virus to limit INFα signalling and hence host defence mechanisms (Christen et al., 2007). Work by Sunahori et al. (2009) suggest that epigenetic methylation of deoxycytosine in the GC rich regions of the PP2Acα promoter inhibit promoter activity by limiting binding of pCREB to CRE. Surprisingly, this does not affect Sp1 binding, despite the close proximity of the Sp1 and CRE motifs (Sunahori et al., 2009). This is interesting as hypomethylation is a key feature of many diseases associated with dysregulation of PP2A expression. For example, in patients with type 2 diabetes, hyperglycaemia causes hypomethylation of the PP2Ac promoter (Tros et al., 2014).

Other transcription factors have also been implicated in the transcriptional regulation of PP2Ac. Using a combined approach of overexpression of a dominant negative form of IKKα and a luciferase reporter gene assay, LPS and macrophage conditioned medium were found to downregulate PP2Ac transcription via a NF-κB-dependent pathway (Tao et al., 2016). DNA-binding protein Ikaros, also decreases PP2Ac expression and abundance by binding to a region in the first intron of PP2Ac following recruitment of histone deacetylase (HDAC1) (Nagpal, Watanabe, Tsao, & Tsokos, 2014). However, it is unclear as to the role of HDAC1 in the transcriptional regulation of PP2Ac, as HDAC1-mediated de-acetylation of Sp1 augments its binding to p300, which increases PP2Ac promoter activity in Hela cells (Chuang & Hung, 2011).

3.2. Key Endogenous Inhibitors of PP2A

3.2.1. SET

SET or inhibitor-2 of PP2A (Fig. 3) is a 277-amino acid protein with a molecular weight of 39 kDa, which was discovered as a translocated gene fused with the CAN gene in acute undifferentiated leukaemia (Von Lindern et al., 1992). It is a potent and selective inhibitor of PP2A that does not inhibit PP1, PP2B or PP2C (Li, Guo, & Damuni, 1995; Li, Makkinje, & Damuni, 1996). Mutational analysis indicates that SET inhibits PP2Ac through binding to both the C (residues 176-277) and N (residues 1-175) terminal domains of PP2Ac (Arnaud et al., 2011; Saito, Miyaji-Yamaguchi, Shimoyama, & Nagata, 1999). Intriguingly, SET has multiple phosphorylation sites, which undergo post-translational modification to regulate its' activity. In acute undifferentiated leukaemia, SET is activated following phosphorylation at Ser^{9} and Ser^{24} (Adachr, Pavlakis, & Copeland, 1994), while phosphorylation of Ser^{171} by protein kinase D2 (PKD2) diminishes its inhibitory action (Irie, Harada, Araki, & Nishimura, 2012).

SET is principally located in the nucleus (Adachr et al., 1994), but can translocate to the cytoplasm in neurones following cleavage at Asn^{175}, yielding C and N terminal fragments (I2CTF and I2NTF). This increases its inhibitory effect on PP2A (Arnaud et al., 2011). While not specifically demonstrated for the I2CTF and I2NTF fragments, SET can bind to heterogeneous nuclear ribonucleoprotein A2 (hnRNPA2) in a cooperative manner to enhance PP2A inhibition and promote cancer cell proliferation (Vera et al., 2006). From a pathophysiological perspective, SET is highly expressed in multiple cancers including leukaemia (Christensen, Chen, et al., 2011) and lung (NSCLC), and is associated with disease progression, metastasis and poor prognosis (Liu et al., 2015).

3.2.2. CIP2A

Cancerous inhibitor of PP2A (CIP2A; Fig. 3) has a molecular weight of 90 kDa (Hoo, Zhang, & Chan, 2002) and is composed of 905 amino acid. It is highly expressed in a plethora of tumours including those of the lung, breast, oesophagus, and gastrointestinal tract (Come et al., 2009; Dong et al., 2011; Khanna et al., 2009; Qu et al., 2012). Like SET, its overexpression is associated with poor prognosis (Barragan et al., 2015; He, Wu, Li, Cao, & Liu, 2012; Khanna et al., 2009). From co-immunoprecipitation experiments we know that CIP2A interacts with PR65 and PP2Ac to prevent holoenzyme formation (Junttila et al., 2007). This has been extended using multiple approaches, including resolution of the crystal structure of CIP2A, to revealed that it forms a homodimer which bind to the B56α and B56γ subunits of PP2A via a conserved N-terminal domain (Wang, Okkeri, et al., 2017). Importantly, prevention of dimerisation or expression of the B56α/γ subunits destabilises CIP2A.

Functionally, the pro-oncogenic effects of CIP2A are multifactorial as it prevents dephosphorylation of c-Myc (Ser^{62}) (Junttila et al., 2007; Khanna, Pimanda, & Westermarck, 2013), stabilises E2F1 (Laine et al., 2013) and inhibits autophagy (Puustinen et al., 2014). Hence, CIP2A is emerging as an important therapeutic target in cancer (Come et al., 2009; Junttila et al., 2007). It is also implicated in the pro-apoptotic effect of bortezomib in triple negative breast cancer (Tseng et al., 2012a).

3.2.3. Alpha-4 (α4)

Alpha-4 (Fig. 3) was initially discovered as a phosphorylated protein in murine B-cells (Kuwahara et al., 1994). It is a 340-amino acid protein with a molecular weight 52 kDa that is widely expressed (Inui et al., 1995). The consensus is that α4 inhibits PP2A activity (Inui et al., 1998; Murata et al., 1997; Nanahoshi et al., 1998), by binding to PP2Ac (Nanahoshi et al., 1998). However, α4 can increase PP2A activity (Nien, Dauphinee, Moffat, & Too, 2007) by stabilising PP2Ac, which aids holoenzyme assembly and protects PP2Ac from proteasomal degradation (Kong, Ditsworth, Lindsten, & Thompson, 2009). This is because α4 provides a binding platform for both PP2Ac and E3 ubiquitin ligase Mid1, which prevents ubiquitination and subsequent degradation of PP2Ac (Mcconnell et al., 2010).

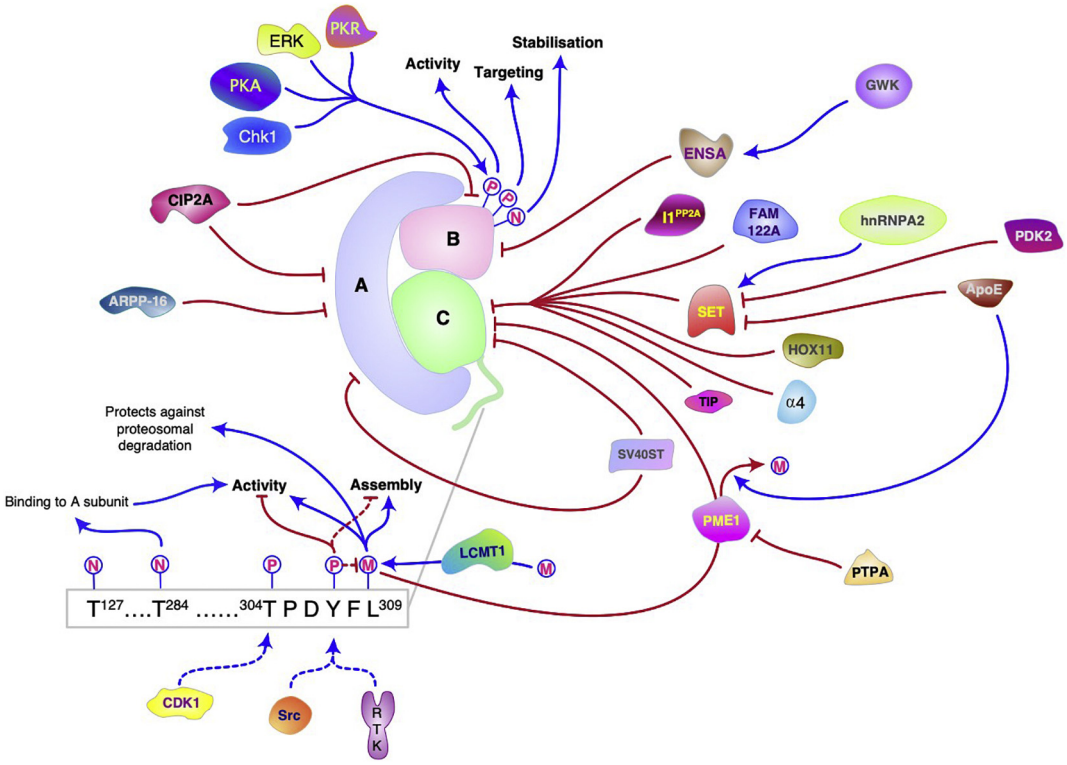

Fig. 3. Diagrammatic overview of regulation of the PP2A holoenzyme through post-translational modification and by endogenous inhibitors. The major site for post-translational regulation is are residues ^{304}TPDYFL^{309} of the C-terminal tail. Phosphorylation of Thr^{307} inhibits catalytic activity, while methylation of Leu^{309} stimulates catalytic activity aids in holoenzyme assembly. The latter is mediated through the opposing actions of LCMT-1 and PME1. Interestingly, nitration of Thr^{284} enhances binding of PP2Ac to the scaffolding subunit. Regarding the interaction of the endogenous inhibitors with the PP2A holoenzyme CIP2A binds to both the scaffolding and regulatory subunits, while SET and α4 interact with the catalytic subunit. *Abbreviations*: ENSA, α-endosulfine; GWK, Greatwall kinase; I1PP2A, Inhibitor 1 of PP2A; PTPA, Serine/threonine-protein phosphatase 2A activator; PME-1, PP2A methylesterase-1; SV40ST, Soluble, monomeric simian virus 40 (SV40) small-t antigen; LCMT-1, Leucine carboxyl methyltransferase 1; RTK, Receptor tyrosine kinases; Src, Proto-oncogene tyrosine-protein kinase; CDK1, cyclin dependent kinase 1; CIP2A, Cancerous inhibitor of PP2A; PKA, Protein kinase A; ERK, Extracellular signal-regulated kinase; PKR, Protein kinase R; FAM122A, family with sequence similarity 122A; TIP, type 2A interacting protein.

3.2.4. Inhibitor 1 of PP2A (I1^{PP2A})

I1^{PP2}A (aka pp32, LANP, PHAP-I, Anp32A and mapmodulin) was initially isolated from lysates of bovine kidney (Li et al., 1995). It was subsequently shown to inhibit PP2A upon binding to PP2Ac (Chen, Wang, Jiang, & Ding, 2013). Functionally, I1^{PP2A} (Fig. 3) is involved in regulation of signal transduction (Vaesen et al., 1994), cell mediated cytotoxicity (Beresford, Kam, Powers, & Lieberman, 1997), tumour suppression (Bai, Brody, Kadkol, & Pasternack, 2001), and microtubule function (Ulitzur, Humbert, & Pfeffer, 1997).

In addition to the above, there are a several other direct and indirect inhibitors of PP2A (Fig. 3). The direct inhibitors include, ARPP-16 (cyclic adenosine monophosphate-regulated phosphoprotein 16 (Andrade et al., 2017), FAM122A (family with sequence similarity 122A) (Fan et al., 2016), ENSA (endosulfine) (Gharbi-Ayachi et al., 2010), TIP (type 2 interacting protein) (Mcconnell, Gomez, Mccorvey, Law, & Wadzinski, 2007), simian virus 40 small-t antigen (SV40ST) (Yang et al., 1991) and HOX11 (homeobox 11) (Kawabe, Muslin, & Korsmeyer, 1997). Whereas, great wall kinase (GWK), hnRNP2A (heterogeneous nuclear ribonucleoprotein A2), ApoE (apolipoprotein E), PTPA and PME1 (PP2A methyl esterase-1) alter PP2A activity indirectly through modulation of the direct inhibitors, or via post-translational modification of PP2A (Fig. 3).

4. Post-translation modification of PP2A

4.1. Phosphorylation of the PP2Ac C-terminal motif

Early reports indicated that receptor and non-receptor tyrosine kinases (EGF, and JAK2) decreases PP2Ac catalytic activity (Chen, Martin, & Brautigan, 1992; Fuhrer & Yang, 1996) as a consequence of tyrosine phosphorylation (Yokoyama, Reich, & Miller, 2001). Subsequent studies, which included mutation analysis, identified Tyr^{307} in the C-terminal tail as the key residue phosphorylated (Longin et al., 2007; Ogris et al., 1997). Phosphorylation of Tyr^{307} has two main effects, 1) it diminishes binding of B55 and B56 regulatory subunits to PP2Ac, and 2) it prevents methylation of Leu^{309} in the conserved ^{304}TPDYFL^{309} motif; both of which interfere with formation of the PP2A holoenzyme (Longin et al., 2007; Nunbhakdi-Craig et al., 2007). Phosphorylation of Tyr^{307} can also reduce PP2A holoenzyme activity by facilitating binding of SET and CIP2A (Lucas et al., 2011; Neviani et al., 2005), and through prevention of PTPA binding (Sents et al., 2017).

Similarly, in an early *in vitro* study, phosphorylation of Thr residues of PP2A by an autophosphorylation-activated protein kinase extracted from bovine kidney, was associated with inhibition of PP2A activity (Guo & Damuni, 1993). Later studies revealed that phosphorylation of Thr^{304} in the C-terminal motif prevented binding of B55 to PP2Acα and hence holoenzyme formation (Gentry et al., 2005; Longin et al., 2007; Nunbhakdi-Craig et al., 2007; Ogris et al., 1997). This would account for the loss of activity in the earlier study by Gou and Damuni.

In contrast, much less is known about the dephosphorylation of PP2A. However, PTPA increases PP2A activity via dephosphorylation of Tyr^{307} in rat hippocampus (Luo et al., 2013), while Tyr^{304} undergoes auto-dephosphorylation (Chen et al., 1992). As the latter is based upon data obtained in the presence of the PP2A inhibitor, okadaic acid, it should be viewed with caution, as okadaic acid inhibits other protein phosphatases, including PP1, PP2B, PP4, and PP6 (Swingle, Ni, & Honkanen, 2007).

4.2. Phosphorylation of PP2A Scaffolding and Regulatory subunits

The scaffolding and regulatory subunits also undergo post-translation modification. Using a combination of MALDI/TOF analysis and site directed mutagenesis, phosphorylation of PR65A at Ser^{303}, Thr^{268} and Ser^{314} was found to disrupt its binding to PP2Ac, and inhibit PP2A–dependent signal transduction (Kotlo et al., 2014). Likewise, in CHO cells, B56 dissociates from PP2Ac upon phosphorylation by ERK (extracellular signal regulated kinase) (Cho & Xu, 2007; Letourneux, Rocher, & Porteu, 2006). Juxtaposed to this, phosphorylation of B56α and PR72 by protein kinase R, protein kinase A or check point kinase 1, enhance PP2A activity and broaden substrate specificity (Margolis et al., 2006; Usui et al., 1998; Xu & Williams, 2000). Lastly, PKA-mediated phosphorylation of B56δ at Ser^{566} increases PP2A activity *in vivo* and *in vitro* (Ahn, Mcavoy, et al., 2007). While unmistakable evidence exists, demonstrating that phosphorylation modulates PP2A holoenzyme activity, further work is required to understand the nuanced effect on holoenzyme assembly, catalytic activity, and substrate specificity.

4.3. Methylation and demethylation

PP2Ac is methylated at Leu^{309} in the conserved ^{304}TPDYFL^{309} motif (Favre, Zolnierowicz, Turowski, & Hemmings, 1994) by leucine carboxyl methyltransferase 1 (LCMT-1), which uses S-adenosylmethionine as the methyl donor (De Baere et al., 1999; Lee & Stock, 1993; Xie & Clarke, 1993). Resolution of the crystal structure of LCMT-1 shows that it has a binding pocket which recognises the C-terminal tail of PP2A (Stanevich et al., 2011). Importantly, methylation of PP2Ac at Leu^{309} has a dual effect. Firstly, it enhances the affinity of PP2Ac for B55 (Longin et al., 2007; Wu et al., 2000; Xu et al., 2008; Yu et al., 2001), and secondly, it increases activity of the PP2A holoenzyme (Favre et al., 1994; Kowluru, Seavey, Rabaglia, Nesher, & Metz, 1996; Leulliot et al., 2004). While methylation is required for binding of the B subunits to PP2Ac, it is not a prerequisite for attachment of B'/PR61 and B″/PR72 subunits (Longin et al., 2007). Although methylation of PP2Ac is generally accepted to increase PP2A activity, an early *in vitro* study failed to show methylation to alter the catalytic activity of purified bovine PP2A, irrespective of whether PP2Ac existed as an A/C subunit dimer (core enzyme) or trimer (De Baere et al., 1999). Nevertheless, resolution of the crystal structure of the LCMT-1 complexed with PP2A shows that activation of the catalytic site of PP2A promotes methylation of its C-terminal motif, which may represent a mechanism for the transmutation of activated PP2A into a substrate-specific holoenzyme (Stanevich et al., 2011).

Juxtaposed to LCMT-1 is protein phosphatase methylesterase-1 (PME-1). Upon binding to PP2A, PME-1- demethylates PP2Ac (Ogris et al., 1997), which removes Mn^{2+} from the active site of PP2Ac to inhibit activity (Longin et al., 2004; Xing et al., 2008). The interaction between PME-1 and PP2Ac is intrinsic upon Arg^{369} (Pokharel et al., 2015). In addition to catalysing demethylation of PP2Ac, PME-1 stabilises the PP2A complex by keeping part of the PP2Ac pool in an inactive state (Longin et al., 2004; Wepf, Glatter, Schmidt, Aebersold, & Gstaiger, 2009). Interestingly, ApoE can inhibit PP2Ac activity via demethylation and disruption of holoenzyme formation (Theendakara, Bredesen, & Rao, 2017), which has important implications in cardiovascular and cerebrovascular disease due to the established role of ApoE in lipid metabolism (Kockx, Traini, & Kritharides, 2018).

How then is the activity of the PP2A holoenzyme restored following demethylation? This is mediated through several mechanisms facilitated by PTPA. Firstly, PTPA may simply displace PME-1 from the PME-1-PP2Ac complex to reactivate holoenzyme activity (Longin et al., 2004). Secondly, PTPA can isomerise Pro^{190} in the active site of PP2Ac (Jordens et al., 2006), which elicits a conformational change in PP2Ac enabling re-association with its regulatory subunits (Hombauer et al., 2007; Longin et al., 2004; Stanevich et al., 2011). Thirdly, PTPA increases methylation and hence activation of PP2Ac which facilitates binding of LCMT-1 to PP2Ac (Guo et al., 2014).

In addition to modulating PP2Ac activity, methylation guides localisation of PP2Ac within a cell. For instance, following activation of A1 adenosine receptors, LCMT-1-mediated methylation of the C-terminal tail of PP2Ac directs it to membrane-rich particulate compartments (Longman, Ranieri, Avkiran, & Snabaitis, 2014). Similarly, LCMT-1, methylated PP2Ac, and Bα are enriched in cholesterol-rich membrane rafts from N2a cells (Sontag, Nunbhakdi-Craig, & Sontag, 2013). While in Arabidopsis, methylated PP2Ac is concentrated in the microsomal fraction rather than the cytosolic fraction, which contains unmethylated PP2Ac.

4.4. Nitration

Nitration is another critical post-translational modification regulating PP2A holoenzyme activity. In endothelial cells, nitric oxide- and superoxide-mediated nitration of PP2Ac inhibits PP2Ac activity and phosphorylation, and is associated with dysfunction of the endothelial barrier (Wu & Wilson, 2009). Although reactive nitrogen species-mediated nitrosylation of PP2Ac is also noted to increase its activity in intestinal and breast cancer cell lines, and in cardiac tissue (Guner et al., 2009; Kohr, Davis, & Ziolo, 2009; Wilson & Yakovlev, 2016; Yakovlev, 2013). However, the nitrosylation site(s) are poorly documented. In a study by Deng et al., Tyr^{127} is nitrosylated following endothelial-mesenchymal transition (Deng et al., 2016), while exposure of purified PP2Ac to peroxynitrite triggers nitration of Tyr^{284} (Ohama & Brautigan, 2010). Importantly, mutation of Tyr^{284} to phenylalanine protects macrophages against peroxynitrite-induced nitration and reduces binding of the scaffolding subunit (Ohama & Brautigan, 2010). Nitrosylation is not restricted to the catalytic subunit as B56δ is nitrated at Tyr^{289}, which prevents PP2A holoenzyme assembly (Low, Loh, Huang, Virshup, & Pervaiz, 2014).

5. Regulation of the PP2A system by microRNA

5.1. Catalytic subunits

MicroRNAs (miRNAs) are small endogenous sequences of RNA which either repress transcriptional or decrease mRNA stability (Bartel, 2004). To date approximately 22 miRNAs (Table 2) are implicated in the regulation of the major components of the PP2A system. A number of reports demonstrate that miR-155, miR-183, miR-429, miR-520h, miR-133a and miR-1246 decrease PP2Ac promoter activity, mRNA expression and protein expression (Belevych et al., 2011; Bott et al., 2017; Guo, Chen, Ji, Mao, & Xie, 2017; Lashine, Salah, Aboelenein, & Abdelaziz, 2015; Qiu et al., 2014). While, other groups show that miR-1 and miR-200c do not alter PP2Ac or PP2Acα protein abundance in adult rat isolated ventricular cardiomyocytes (Terentyev et al., 2009) or in oesophageal squamous cell carcinoma (Hamano et al., 2011) respectively. From a clinical perspective, low expression of miR-155 correlates to high levels of PP2Ac mRNA expression in PBMCs from patients with systemic lupus erythematosus or familial Mediterranean fever (Lashine et al., 2015), while miR-1246 induces a pro-inflammatory response through direct targeting of PP2Ac and PKA in mesenchymal stem cells (Bott et al., 2017). Interestingly, miR-429 protect osteoblasts against dexamethasone induced cell death and apoptosis in a PP2Ac dependent manner (Guo et al., 2017).

5.2. Scaffolding and regulatory subunits

Regarding the PP2A scaffolding subunits, miR-200c and miR-587 decrease expression of the PPP2R1B to confer chemoresistance in oesophageal squamous cell carcinoma and colorectal cancer (Hamano et al., 2011; Zhang, Talmon, & Wang, 2015). While there does not appear to be any experimental evidence supporting miRNA regulation of

Table 2
miRNAs implicated in regulation of key components of the PP2A system

Subunit	Gene Target	miRNA	Tissue/model	Target and Effect	Notes	References
Catalytic	PPP2AC	miR-1	Adult rat isolated ventricular myocytes	↔ PP2Ac protein	miR-1 ↔ on PP1C protein	(Terentyev et al., 2009)
		miR-155	PBMCs	↓ PP2Ac mRNA	↓ miR-155 correlates to ↑ PP2Ac mRNA in PBMCs from patients with SLE or Familial Mediterranean Fever	(Lashine et al., 2015)
		miR-429	OB6, hFOB1.19	↓ PP2Ac mRNA/protein ↔ PP2A-A	Targets 3'-UTR of PP2Ac mRNA	(Guo et al., 2017)
		miR-520h	Human breast and cervical cancer cell lines	↓ PP2Ac promoter ↔ PP2Ac mRNA ↓PP2Ac protein /phosphatase activity	Targets 3'-UTR of PP2Ac mRNA	(Su et al., 2010)
		miR-200c	Oesophageal squamous cell carcinoma	↔ PP2Acα protein		(Hamano et al., 2011)
		miR-183	ACHN, A498 renal tumour biopsies	↓ PP2Acα and PP2Acβ protein / promoter activity	Regulates cell growth and metastasis in renal cancer	(Qiu et al., 2014)
		miR-133a	CHO	↓ PP2Acα and PP2Acβ promoter activity	miRNA overexpression	(Belevych et al., 2011)
		miR-1 miR-133a	Rat cardiomyocytes	↓ PP2Ac protein		
		miR-1246	Mesenchymal stem/stroma cells	↓ PP2Acβ mRNA / promoter activity	Induces pro-inflammatory response by direct targeting of PP2Ac and PKA	(Bott et al., 2017)
Scaffold	PPP2R1B	miR-200c	Oesophageal squamous cell carcinoma	↓ PP2A-Aβ protein	↑ miR-200c associated with chemoresistance	(Hamano et al., 2011)
		miR-587	HCT116; GEO cells; Colorectal cancer biopsy	↓ PP2A-Aβ protein	Confers chemoresistance in colorectal cancer	(Zhang et al., 2015)
	PPP2R2A	miR-31	Murine and human lung cancer cell lines	↓ PPP2R2A mRNA ↓ promoter activity	miR-31 and PPP2R2A expression inversely related in murine and human lung cancer	(Liu et al., 2010)
Regulatory		miR-195	Murine and human lung cancer cell lines	↓ PP2ABα expression	Represses specific tumour suppressors in lung cancer	(Liu et al., 2016)
		miR-222	Hep3B and HKCI-9	↓ PP2A-Bα protein, promoter activity		(Wong et al., 2010)
		miR-17 + miR-20	DLBCL cells & DHL4 cells	↓ PPP2R2A promoter activity and mRNA		(Jablonska et al., 2017)
	PPP2R2D	miR-133b	HepG2, QGY7701, MHCC97H, HCC97L, Hep3B, Huh7	↓ PP2A-Bδ protein and mRNA	miR-133b binds to 3'UTR enhancing sensitivity of HCC to chemotherapy	(Zhuang et al., 2016)
	PPP2R5A	miR-1	Adult rat isolated ventricular myocytes	↓ PP2A-B'α protein	miR-1 enhances cardiac excitation contraction coupling by ↓ PP2A-Bα abundance and altering cellular localisation of PP2Ac	(Terentyev et al., 2009)
		miR-155 miR-31	Murine macrophages	↓ PP2A-B'α mRNA ↓ PP2A-B'α promoter activity	Decreases PP2A activity limiting autophagy	(Holla et al., 2014)
		miR-1 miR-133	Canine heart failure Cardiomyocytes	see note	miR-1 and miR-133 expression associated with decrease protein levels of B56α/δ and lower PP2A activity	(Belevych et al., 2011)
	PPP2R5C	miR-27a	HBELH		B56γ KO ↑ miR-27a mRNA B55α KO ↓ miR-27a mRNA	(Wang et al., 2011)
		miR-183	ACHN, A498 renal tumour biopsies	↓ PP2A B56γ protein / promoter activity	Regulates cell growth and metastasis in renal cancer	(Qiu et al., 2014)
	PPP2R5D	miR-9	HEK293 Pulmonary macrophages	↓ PPP2R2A ↓ PPP2R5D promoter activity ↓ PP2A-B'δ	Inhibition of miR-9 ↑ PP2A activity	(Li et al., 2015)
		miR-1 miR-133	Canine heart failure Cardiomyocytes	see note	miR-1 and miR-133 expression associated with decrease protein levels of B56α/δ and lower PP2A activity	(Belevych et al., 2011)
	PPP2R5E	miR-19	Lymphocytes	↓ PPP2R5E mRNA, promoter activity, protein	miR-19 ↓ PTEN mRNA, promoter activity, and protein	(Mavrakis et al., 2010)
			DLBCL cells & DHL4 cells	↓ PPP2R5E promoter activity and mRNA		(Jablonska et al., 2017)
Other	CIP2A	miR-218	A375 SK-MEL-2 cells	↓ CIP2A promoter, mRNA, and protein	Suppresses proliferation, migration, invasiveness, and the cell cycle	(Wei et al., 2014)
		miR-375	HEK293 CAL27	↓ CIP2A promoter activity, ↓ mRNA, ↓ protein	Inverse correlation between miR-375 and CIP2A in oral cancer and NCI-60 cells but not in multiple head and neck cancer cell lines	(Jung et al., 2013)
		miR-383-5p	A549 H1299	↓ CIP2A protein, ↓ mRNA, ↓ promoter activity	miR-383-5p inversely related to CIP2A expression in lung adenocarcinoma	(Zhao et al., 2017)
	SET	miR-199b	JAR BeWo	↓ SET protein, ↓ promoter activity	miR-199b associated with ↑ SET in choriocarcinoma	(Chao et al., 2010)
	α4	miR-34b	L02RT-AFB$_1$, SMMC and A549 vrlld	↓ α4 expression and promoter activity	α4 overexpressed in human primary hepatocellular carcinoma, lung, and breast cancer samples	(Chen, Lai, et al., 2011)
	PME-1	miR-195	Rat hippocampal and cortical regions	↑ PME promoter activity		(Liu et al., 2016)

PPP2R1A, a transcriptome/targetome analysis reveals that PPP2R1A functions as a regulatory network node for miR-16 (Pan, Guo, Sun, Fan, & Fang, 2014).

Several studies have highlighted a role for miRNA in modulating expression of the B55 (PPP2R2) and B56 (PPP2R5) regulatory subunits. In murine and human lung cancer cell lines, Liu et al. demonstrated an inverse relationship between miR-31 and PPP2R2A expression (Liu et al., 2010). In the same study the authors clearly revealed that miR-31 down-regulates expression of PPP2R2A mRNA through direct binding to its promoter region. More recently miR-17 and miR-20 have been shown to inhibit PPP2R2A promoter activity, and hence PPP2R2A mRNA expression in DLBCL and DHL4 cells (Jablonska et al., 2017). In complementary experiments, silencing of miR-195 and miR-222 were noted to increase PP2A-Bα protein abundance, mRNA expression and restore promoter activity in Hep3B and HKCL-9 cells and in rat hippocampi (Liu et al., 2016; Wong et al., 2010). Regarding PP2R2D, expression of miR-1 and miR-133 is associated with lower levels of PP2A-Bδ (PPP2R2D) in ventricular cardiomyocytes from a canine model of heart failure (Belevych et al., 2011). In more recent work, miR-133b was shown to bind to the 3'-UTR of PPP2R2D, and decrease transcription in hepatic carcinoma cell lines; an effect associated with chemosensitisation (Zhuang et al., 2016).

There is also evidence showing miRNA regulation of the B56 regulatory subunit. In ventricular cardiomyocytes, overexpression of miR-1 decreases expression of PPP2R5A and hyper-phosphorylates the ryanodine receptor (RyR2) (Terentyev et al., 2009). While in a canine model of heart failure, overexpression of miRNA-1 and miRNA-133 are associated with downregulation of PPP2R5A and inhibition of PP2A activity (Belevych et al., 2011). In both studies arrhythmogenesis is increased. In PBMC, miR-155 and miR-31 mediated downregulation of PP2A-Bα expression attenuates PP2A activity to limit INFγ-induced autophagy (Holla, Kurowska-Stolarska, Bayry, & Balaji, 2014). In pulmonary macrophages, MiR-9 regulates steroid resistant airway hyperresponsiveness by decreasing PP2A-B'δ abundance and promoter activity (Li et al., 2015). Similarly, miR-19 decreases PPP2R5E mRNA, protein, and promoter activity in diffuse large B cell lymphoma cells and in lymphocytes (Jablonska et al., 2017; Mavrakis et al., 2010).

5.3. Other components

Studies focussing on experimental models of cancer demonstrate that miRNAs have a key role in regulating the expression of several endogenous inhibitors of PP2A. For instance, miR-218, miR-375 and miR-383-5p decrease CIP2A promoter activity, mRNA expression and protein abundance in skin, oral and lung cancer cell lines (Jung et al., 2013; Wei et al., 2014; Zhao et al., 2017). Interestingly, while there is an inverse relationship between miR-375/miR-383-5p and CIP2A expression in oral and lung cancer this is not noted in multiple head and neck cancer cell lines (Jung et al., 2013). Regarding other endogenous inhibitors of PP2A, overexpression of miR-199b decreases SET promoter activity, expression, and abundance in choriocarcinoma cell lines, (Chao et al., 2010), while α4 expression is attenuated by miR-34b (Chen, Lai, et al., 2011).

Post-translational modification of the PP2A holoenzyme is an emerging target under miRNA regulation. In a study by Liu et al., knockdown of miR-195 decreased expression of PME-1 (Liu et al., 2016), which was manifest as a decrease in methylation of PP2Ac at Leu^{309} in rats with chronic brain hypoperfusion. While miRNA clearly modulates the expression of different components in the phosphoprotein phosphatase system, a reciprocal relationship also exists. In bronchial epithelial cells, knockdown of B56γ upregulates miR-27a (Wang et al., 2011). This is attributed to increased binding of phosphorylated CREBPα to the promoter region of miR-27a, as occurs with miR-122 (Zeng et al., 2010). In another study, silencing of B55α increases expression of miR-191-5p and attenuated expression of miR-142-3p in AML cells (Ruvolo et al., 2014). The latter paradigm is supported by another study demonstrating PP2A-gain of function to increase miR-29 through activation of histone deacetylase 4 (HDAC4) in fibroblasts from patients with idiopathic pulmonary fibrosis (Khalil et al., 2015). Clearly a complex inter-relationship between PP2A and miRNA exists, warranting further investigation.

6. ROS-mediated regulation of PP2A and oxidative stress (Table 3)

Low levels of reactive oxygen species (ROS) are physiologically important in cell signalling, and biosynthetic pathways implicated in cell growth, migration, and immune activation. However, excessive levels damage cellular proteins, lipids, and DNA, and are implicated in numerous diseases such as cardiovascular disease and cancer. Pathophysiologically, defective mitochondrial function, increased NADPH oxidase (NOX) activity, and uncoupling of nitric oxide synthase (NOS), are the main causes of excessive generation of H_2O_2, superoxide ($O_2^{\bullet-}$), hydroxyl ($OH^{\bullet-}$), nitric oxide ($NO^{\bullet}$) and peroxynitrite ($ONOO^-$) radicals (Kanaan & Harper, 2017) (Fig. 4). To compound this, levels of cellular antioxidants including NADH and NADPH, superoxide dismutase (SOD), glutathione and catalase, are frequently depleted. Divergent views exist with respect to the effect of ROS on PP2A, with ROS being reported to increase, decrease, or have no effect (Table 3).

So, what is the evidence that ROS and RNS increase PP2A activity? Firstly, the antioxidant and free radical scavenger, N-acetylcysteine, attenuates dephosphorylation of PP2A at Tyr^{307} in cancer associated fibroblasts following exposure to IGF2 post radiation (Wang, Gan, et al., 2017) and reduces licochalcone A induced PP2A abundance in Huh7 and HepG2 cells (Niu et al., 2018). Similarly, superoxide dismutase and phenanthroline (an iron chelator) reduce $O_2^{\bullet-}$ mediate PP2A expression and abundance following intermittent hypoxia in PC12 cells (Chen et al., 2014). Consistent with this, other groups have shown NOX inhibition to prevent ROS-mediated nitrosylation of PP2Ac, and PP2Ac catalytic activity (Han et al., 2010; Wilson & Yakovlev, 2016; Wu & Wilson, 2009). This extends data from an *in vivo* study showing NOX activation and uncoupling of endothelial nitric oxide synthase (eNOS) to increase levels of 3-nitrotyrosine (a product of peroxynitrite), and PP2A activity following caecal ligation and puncture; effects which were prevented by intravenous injection of ascorbate (200 mg/kg body wt) (Zhou, Kamenos, Pendem, Wilson, & Wu, 2012).

Several reports have established H_2O_2 to increase PP2A activity (Cicchillitti, Fasanaro, Biglioli, Capogrossi, & Martelli, 2003; Jiang et al., 2017; Maalouf & Rho, 2008; Sen, Kawahara, & Chaudhuri, 2012). This is likely due to increased methylation of PP2Ac and changes in its colocalisation with B56α as occurs in ventricular myocytes (Deshmukh, Blunt, & Hofmann, 2007). Further support comes from the observation that glutathione depletion increases PPP2CA mRNA, but not protein abundance in a N-acetylcysteine sensitive manner in murine fibroblasts (Reid et al., 2013). Interestingly, glutathione depletion increased B55α but not B55β, B55γ, B55δ or B56α abundance (Reid et al., 2013). Finally, in primary mouse osteoblasts, 4-HNE, a lipid peroxidation product increases PP2A activity without altering PP2Aa, PP2AB′ or PP2Ac abundance (Huang, Lv, & Wang, 2015). Together, these data provide good evidence that free radicals increase PP2A activity and transcription.

However, there is equally good data to the contrary. In an early report, H_2O_2 was observed to decrease the okadaic acid sensitive component of protein phosphatase activity by 80-85% independently of altering PP2C or PP1 abundance in Caco-2 cells (Rao & Clayton, 2002). In keeping with this, more recent work has shown H_2O_2 and glucose oxidase, which catalyses the oxidation of glucose to H_2O_2 (Fig. 4), decrease PP2A and total PP1/PP2A activity in human breast cancer cells and ARPE-19 cells (Antony, Lukiw, & Bazan, 2010; Sen et al., 2012). Intriguingly, Antony et al. established that knockdown of PP2Acα exacerbated oxidative stress induced apoptosis (Antony et al., 2010).

Several other studies also implicate ROS in the inactivation of PP2A. In human fibroblasts, low dose radiation, which reduces cellular glutathione levels and increases mitochondrial ROS inactivates PP2A

Table 3
ROS and RNS modulation of PP2A

ROS/RNS Species/stimulus	Cell Line	Effect on PP2A	Additional Notes	Implication	Reference
H_2O_2 trophic factor deprivation	661w cells	↓mPP2Ac ↑ pPP2Ac	Prevented by N-acetylcysteine	Upregulation of PI3K / Akt and ERK survival pathways	(Finnegan et al., 2010)
H_2O_2	Caco2	↓ PP2A activity ↔ PP2C or PP1	Reversed by glutathione	Glutathionylation may mediate H_2O_2 inhibition of PP2A	(Rao & Clayton, 2002)
H_2O_2 Glucose oxidase	C2C12	↑ thiol oxidation of PP2A and PTEN			(Tan et al., 2015)
	32D-BCR-ABL1	↓ PP2A activity	Imipramine blue restores PP2A activity in 32D-BCR-ABL1	NADPH oxidase blockade may be beneficial in eradication of CML	(Laidlaw et al., 2016)
H_2O_2/TNFα	ARPE-19	↓ Total PP1/ PP2A activity	Knockdown of PP2Acα increases stress induced apoptosis	NPD1 increases association of PP2A/Cα with S62Bcl-xL as well as total Bcl-xL	(Antony et al., 2010)
H_2O_2	MDA-MB-468 MCF7	↓PP2A	Reversed by DTT	↑ phosphorylation of Akt and ERK1/2	(Sen et al., 2012)
H_2O_2	PC12 SH-SY5Y	↓ PP2A ↓ PP5	Reversed by N-acetylcysteine	Overexpression of PP2A/PP5 prevented H_2O_2 activation of ERK1/2, JNK p38 and cell death	(Chen et al., 2009)
LPS/INFγ	VSMC (rat)	↓ mPP2A ↑ pPP2A	Reversed by 2,2,5,7,8-pentamethyl-6-hydroxychromane	PMC-mediated inhibition of NF-κB activity occurs via ROS-PP2A-p65 signalling	(Hsieh et al., 2014)
X-ray	TIG3 MCR5	↓ PP2A activity	Reversed by N-acetylcysteine	↑ pAkt	(Shimura et al., 2016)
Cadmium	Rat brain	↓ PP2Ac, PP2A-A, PP2A-B	PP2Ac reversed by nobiletin	↓ PP5 and activation of JNK and ERK1/2 signalling	(Qu et al., 2018)
Fusarochromanone	COS7	↓PP2A-A, ↔ PP2A-B, ↔PP2Ac, ↑demPP2A, ↑pPP2A	PP2A effect reversed by N-acetylcysteine	PP2A effect associated with JNK activation; ↓ PP5; Overexpression of PP2A or PP5 partially reverse fusarochromanone activation of JNK	(Gu et al., 2015)
Intermittent hypoxia	Rat brain	↓ PP2A abundance & activity ↔ PP2C, PP4, PP5, PP6	PP2A effect reversed by antioxidants		(Raghuraman et al., 2009)
Morphine	H9c2	↓ PP2A activity	Prevented by N-(-2-mercaptopropionyl) glycine	OA mimicked morphine induced activation of Akt; Morphine cardioprotective via inhibition of PP2A	(Xu et al., 2011)
Tertbutyl hydroperoxide, 2,2'-azobis (2-amidopropane) dihydrochloride	HEK293T mice	↓ PP2A activity ↑ pPP2Ac ↔ PP2Ac	PP2A inactivation activates NFκB		(Jin Jung et al., 2013)
H_2O_2	*in vitro* biochemical assay	↔ PP2Ac	↔ PP2B ↓ PTP1		(Denu & Tanner, 1998)
H_2O_2	HepG2	↔ PP2Ac		H_2O_2 increases association of PP2Ac with HMG-CoA reductase	(Pallottini et al., 2007)

Abbreviations: Akt, protein kinase B; ERK, extracellular signal related kinase; JNK, Jun N-terminal kinase; NADPH, reduced nicotinamide adenine dinucleotide phosphate; OA, okadaic acid; PI3K, phosphoinositide 3 kinase.

(Shimura et al., 2016). Also, intermittent hypoxia a well-documented stressor associated with ROS generation decreases PP2A abundance and activity independently of altering PP2C, PP4, PP5 or PP6 abundance in dorsal and ventral medulla (Raghuraman, Rai, Peng, Prabhakar, & Kumar, 2009). Although ROS decrease the abundance of PP5 as well as PP2Ac in COS7 cells and rat brain (Gu et al., 2015; Qu et al., 2018). Importantly, the effects of ROS on the PP2A holoenzyme are not restricted to the catalytic subunit as nobiletin and N-acetylcysteine prevent cadmium and fusarochromanone (a mycotoxin) from reducing PP2A-A and PP2A-B, abundance in COS7 cells and rat brain (Gu et al., 2015; Qu et al., 2018). This may be subunit specific, as H_2O_2 down regulates PP2A-A but not PP2A-B abundance in PC12 cells and neurones, an effect reversed by N-acetylcysteine (Chen, Liu, Yin, Luo, & Huang, 2009). Finally, Laidlaw recently demonstrated that imipramine blue, a novel NADPH oxidase inhibitor reactivates PP2A in leukaemia cells (Laidlaw et al., 2016).

Two principle mechanisms have been put forward to explain how ROS modulate the PP2A holoenzyme, 1) demethylation/phosphorylation and 2) reversible oxidation of thiol groups. In vascular smooth muscle, kidney and neuronal cells lines, ROS-mediated inhibition of PP2A activity is accompanied by demethylation, and phosphorylation of PP2Ac at Tyr^{307} (Chen et al., 2009; Finnegan, Mackey, & Cotter, 2010; Gu et al., 2015; Hsieh, Hsiao, Hsu, Wang, & Sheu, 2014). However, H_2O_2 may also inhibit phosphatase activity through reversible oxidation of thiol groups (Barrett et al., 1999; Switzer et al., 2009). The inhibitory effect of H_2O_2 on PP2A activity, appears to be more dependent on thiol oxidation, since the methylation and phosphorylation state of PP2Ac following exposure to H_2O_2 are unaffected by N-acetylcysteine (Chen et al., 2009). This is important as ROS may crosslink PP2Ac to other proteins in close proximity, due to the formation of novel intermolecular disulphide bonds as occurs in rat cerebral cortex (Foley, Petro, Stredny, & Coppa, 2007). Crucially, cysteine residues are one of the key ROS 'receptors' within cells that sense redox signals triggering the generation of sulphonic acid (R-SOH) or cysteine disulphide bonds (S-S) from cysteinyl thiols (R-SH). These post-translational modifications also have the ability to alter the conformation and/or activity of redox sensitive proteins (Finkel, 2012). This appears to be concentration dependent, as high levels of glucose oxidase-generated H_2O_2 or H_2O_2 *per se* increase oxidation of thiol groups within PP2Ac by 247% and 768% respectively, but have no effect at lower levels (Tan, Shavlakadze, Grounds, & Arthur, 2015). The 'thiol oxidation' hypothesis is further supported by work showing that pharmacological inhibition and genetic knockdown of SOD1 inhibits assembly of the PP2A holoenzyme and subsequent dephosphorylation of Bcl-2 at Ser^{70} by interfering with recruitment of PP2Ac and PP2A-A to Bcl-2 (Low et al., 2014).

Despite the above, sporadic reports have materialised to indicate that PP2A is resistant to oxidative stress. For instance, glucose oxidase and $O_2^{\bullet-}$ have no effect on PP2A abundance in rat and mouse kidney, and/or PP2A activity in SK-N-SH neuroblastoma cells (Sommer, Coleman, Swanson, & Stemmer, 2002; Zhang et al., 2008). Also, short

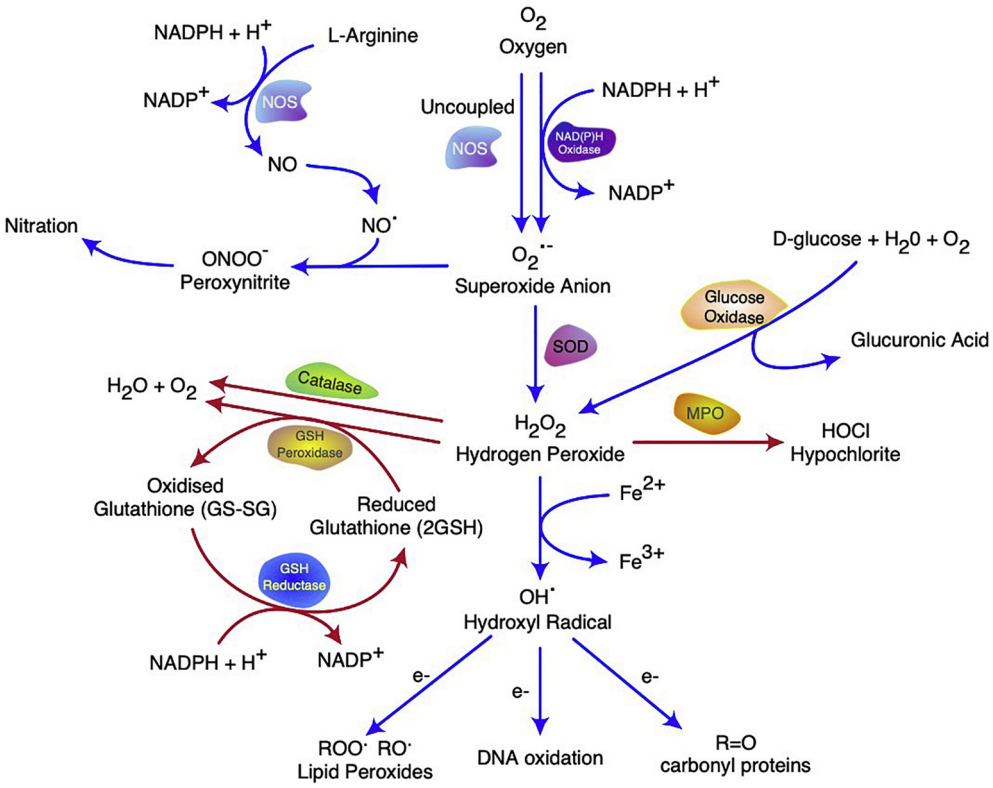

Fig. 4. Overview of free radical generation and metabolism. Blue arrows depict processes contributing to free radical generation, while red arrows represent enzymatic pathways involved in free radical scavenging. Abbreviations: MPO, myeloperoxidase; GSH, glutathione; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NOS, nitric oxide synthase; SOD, superoxide dismutase.

term exposure to H_2O_2 (1 h) does not affect PP2A abundance in HepG2 (Pallottini et al., 2007), supporting an earlier observation that PP2A is resistant to oxidants (Denu & Tanner, 1998). On the flip side, PP2A activity is resistant to H_2S (reducing agent) in SH-SY5Y cells (Xie et al., 2014).

So how do we account for the divergent views on ROS-mediated regulation of PP2A? As already mentioned, this may be dependent on the concentration ROS or its nature (H_2O_2 vs $^{\bullet}O_2^-$), or variation in the sensitivity of different tissues/cells to ROS. Importantly, several of the commercial antibodies used are raised against a C-terminal epitope of PP2Ac. If the C-terminal motif is methylated at Leu^{309} this can sterically interfere with antibody binding, which would give a false impression that protein abundance is decreased. Unfortunately, the issue is complicated further as there is cross talk between the PP2A system and that of ROS. For instance, silencing of CIP2A increases NADH/NAD^+ ratio and ROS production in cancer cells (Peng, Lei, Chai, Chan, & Zhang, 2015), while okadaic acid inhibits catalase and glutathione peroxidase activity in glial cells (Ferrero-Gutierrez, Perez-Gomez, Novelli, & Fernandez-Sanchez, 2008). Interestingly, okadaic acid also reduces cellular content of glutathione and elevates oxidised glutathione, thus potentiating H_2O_2-mediated toxicity (Ferrero-Gutierrez et al., 2008). It is evident that this is a field requiring further research, particularly regarding how ROS modulate holoenzyme assembly, and expression/function of the regulatory and scaffolding subunits.

7. Ischaemia-reperfusion injury and ischaemia: role of PP2A (Table 4)

Ischaemia-reperfusion injury is a significant contributor to morbidity and mortality associated with myocardial infarction, ischaemic stroke, and acute kidney injury (Eltzschig & Eckle, 2011). At a cellular level, ischaemia-reperfusion triggers a sequence of events culminating in tissue injury through generation of ROS, as a consequence of oxidation of lipids, proteins, and nucleic acids (Sun et al., 2018). This is compounded by the rapid depletion of cellular antioxidants within minutes of reperfusion (Katz, Callaway, Kagan, & Kochanek, 1998).

Numerous reports over the last 20 years (Table 4) have focussed on the role of PP2A in ischaemia-reperfusion injury of the brain and heart. In a rat model of forebrain ischaemia-reperfusion, PP2A activity is decreased (Martin De La Vega, Burda, Toledo Lobo, & Salinas, 2002; Zhu et al., 2012), consistent with demethylation of PP2Ac in areas most susceptible to ischaemic damage (Martin De La Vega et al., 2002). The observed decrease in PP2A activity may also reflect downregulation of PP2Ac at the protein level (Jackson et al., 2013; Yi & Simpkins, 2008; Zhou, Qi, & Chen, 2017; Zhu et al., 2012). In support of this, PP2A activity and PP2A subtype A, but not PP2A subtype B, abundance are decreased in a rat isolated heart model of ischaemia-reperfusion (Penna et al., 2011; Xu, Kim, & Huh, 2014). However, as alluded to earlier, several commercially available PP2Ac antibodies are raised against a C-terminal epitope of PP2Ac, which if methylated at Leu^{309} can sterically interfere with antibody binding, thus making interpretation of the experimental data more difficult. With this caveat in mind, the decrease in PP2Ac abundance may be due to increased production of free radical, as ischaemia-reperfusion increases malondialdehyde and NO levels, and decreases antioxidant capacity (GSH and superoxide dismutase) in a mouse model of middle cerebral artery occlusion (Zhou et al., 2017). Interestingly, the neuroprotective effect of EGb761 (antioxidant from Ginkgo Biloba) (Zhou et al., 2017), β-oestradiol (Yi & Simpkins, 2008) and simvastatin (Zhu et al., 2012) is associated with amelioration of ischaemia-reperfusion-mediated downregulation of PP2A. Importantly, decreased PP2A activity/abundance activates ERK1/2 signalling (Ho, Logue, Callaway, & Defranco, 2007; Martin De La Vega et al., 2002), a key pathway underpinning ischaemia-reperfusion injury (Alessandrini, Namura, Moskowitz, & Bonventre, 1999; Namura et al., 2001). This has been extended to show that the decrease in PP2A activity is associated with increased phosphorylation of Akt (Ser^{473}), ERK1/2

Table 4
Effect of Ischaemia-reperfusion and pre/post-conditioning on PP2A

Cell line/Tissue	Model	Protocol	PP2A Response	Additional Notes	Implication	Reference
Rat Brain	Cardiac arrest	8 / 30 min	OA (10 nM) inhibited ERK activation	ERK phosphatase reversibly inhibited in cortex but not hippocampus	Tyrosine phosphatases and DUSP also involved (orthovanadate)	(Ho et al., 2007)
Rat Brain	4 vessel occlusion	carotid occlusion 30 min/15 min-2 h	↓ PP2A Activity ↓ PP2Ac Methylation	demethylated PP2Ac highest in areas susceptible to ischaemic damage	↑ pERK 1/2	(Martin De La Vega et al., 2002)
Rat Brain	tMCAO	1 h / 23 h	↓ PP2A abundance	↓ PP1, ↓ PP2B abundance	Oestradiol neuroprotective – blocks phosphatase degradation	(Yi & Simpkins, 2008)
Rat Brain	MCAO	1 h / 24 h	↓ PP2A activity and abundance	reversed by simvastatin	Simvastatin decreases infarct size through an increase in PP2A	(Zhu et al., 2012)
Rat Brain	Cardiac arrest	6 min / 1-24 h	↓ PP2Ac	↓ PP1α ↓ PTEN		(Jackson et al., 2013)
Mouse	MCAO	60 min / 5 days	↓ PP2A abundance	↓ GSH, ↓ SOD ↑ MDA, ↑NO, ↑ NOX1,2,4, ↑ NAPDH	EGb761 (antioxidant) reversed effect on PP2A and afforded neuroprotection	(Zhou et al., 2017)
Rat brain	Vertebral occlusion	carotid occlusion 10 min / 1 h	↑ PP2A activity	Partially inhibited by TPCK but not Fumo B	Ceramide regulates PP2A activity	(Tian et al., 2009)
Rat Brain	4 vessel occlusion	Carotid occlusion 10 min / 10 min – 24 h	Transient ↑ PP2A activity (1 h) and ↓ pPP2Ac ↑ PP2Ac abundance > 6h ↑ pPP2A Tyr^{307}	Src inhibits PP2A through phosphorylation of Tyr^{307}- biphasic response	↓ PP2A activity activates ERK and CREB/ERα	(Hu et al., 2009)
Rat isolated Heart		I/R	↑ PP2A abundance	↑ PP1B abundance, reversed by simvastatin		(Szobi et al., 2016)
Mouse Heart	LAD occlusion	60 min/ 24 h	↑ PP2A activity	↔ PP1 activity; ↔ PKA activity	PP2A mediated dephosphorylation of phospholamban impairs Ca^{2+} cycling	(Kronenbitter et al., 2018)
Rat Brain	Vertebral arteries occluded	Carotid occlusion 20 min/2 h-7d	↔ PP2A activity	Multiphase ↓ in PP2B activity	PP2B contributes to neuronal injury	(Morioka et al., 1999)
Rat Brain	Cardiac arrest	2-8 min/2h-4 weeks	↔ PP2A activity ↔ pPP2A/PP2A abundance	↔ GSK3β or pGSK3β abundance; ↓ catalase abundance	GSK3β and PP2A not involved in dephosphorylation of pTau	(Majd et al., 2016)
Rat Isolated heart	Ischaemia I/R	20 min 20 min / 60 min	↔ PP2A (no stats) under either condition	↓ pPTEN by ischaemia, normalised on reperfusion		(Zhou et al., 2014)
Rat isolate heart ± chronic Kidney disease		25 min/ 5 min	↔ B55α abundance ↔ pPP2Aα or PP2Ac abundance; ↔ PPP2R2 (A, B, C, and D) mRNA	CKD group I/R ↓ B55α, ↔ PP2AA or PP2Ac	↓ B55α increases infarct size	(Tobisawa et al., 2017)
Rat isolated heart		30 /120 min 10s x 5 I/R post-conditioning	↓ PP2Aα ↔ PP2Aβ			(Penna et al., 2011)
Rat isolated Heart		30 min/2 h post conditioning 6 x 10s	Post conditioning ↓ PP2A activity	Zn^{2+} dependent	Zn^{2+} mediated cardioprotection post conditioning involves ↓ PP2A	(Xu et al., 2014)
Mouse hippocampal slices	OGD 95% N_2, 5% CO_2	1 h reperfusion	↓ PP2A activity which recovers on reperfusion	↓ PP1 activity		(Hedou et al., 2008)
Rat Brain	MCAO	Ischaemia 24 h	↓ PP2A	oestradiol prevents injury induced ↓ PP2A	Oestradiol mediated neuroprotection involves PP2A	(Sung et al., 2010)
Rat Brain	MCAO	Ischaemia 24 h	↓ PP2A subunit B abundance and mRNA	↓ PP2A subunit B prevented by melatonin, ferulic acid (antioxidants)		(Koh, 2011, 2012, 2013)
Rat Brain	MCAO	Ischaemia 24 h	↓ PP2A abundance ↑ GSK3β abundance	partially reversed by acupuncture	Acupuncture neuroprotective	(Luo et al., 2014)
Rat Brain		Ischaemia 24 h	↓ PP2A subunit B mRNA & abundance	reversed by curcumin (antioxidant)	Curcumin neuroprotective	(Shah et al., 2015)
Rat Kidney NRK-52E	Mineral oil	Ischaemia 3 h	↑ PP2A B56 ↑ PP2A activity	↓ PKCα; PP2A KO protective; over expression B56α ↑ cell death; over expression of B55 ↔ cell death; OA (10 nM) protective	PP2A/B56α mediates ischaemic cell death	(Tsao et al., 2007)
Rat hippocampal neurones	OGD	95% N_2 / 5% CO_2 2 h	↑ PP2A abundance	PP2A abundance normalised by NaHS	↓ pERK 1/2 ↔ PKA ↑ MDA, NO, H_2O_2 ↓ SOD, GSH	(Yu et al., 2017)
Rabbit isolated Heart, cardiomyocyte		Global ischaemia, and preconditioning	Global ischaemia ↓ PP2A activity	Global ischaemia ↓ PP1 activity; Preconditioning ↔ PP1 or PP2A activity in myocytes/biopsies vs unconditioned	Fostriecin protective in myocytes and isolated heart comparable to preconditioning	(Weinbrenner et al., 1998)

Table 4 (*continued*)

Cell line/Tissue	Model	Protocol	PP2A Response	Additional Notes	Implication	Reference
Rat isolated Heart	Ischaemia, preconditioning, and I/R	20 min 1x 5min /5 min 3 x 5min /5min	↔ Ser/Thr phosphatase activity	Cantharidin/OA effect dependent upon when administered		(Fan et al., 2010)

Abbreviations: CDK, chronic kidney disease; ERK, extracellular signal related kinase; FumoB, fumonisin B1; GSH, glutathione; GSK3β, glycogen synthase kinase 3β; I/R, ischaemia reperfusion; LAD, left anterior descending coronary artery; MDA, malondialdehyde; MCAO, middle cerebral artery occlusion; NADPH, nicotinamide adenine dinucleotide phosphate; NOX, nitric oxide synthase; OA, okadaic acid; OGD, oxygen glucose deprivation; PKA, protein kinase A; SOD, superoxide dismutase; Src, Proto-oncogene tyrosine-protein kinase; TPCK, N-tosyl-phenylalanine chloromethyl-ketone.

(Thr^{202}/Tyr^{204}), and glycogen synthase kinase-3β (GSK-3β) (Ser^{9}) (Xu et al., 2014).

To counterbalance this view, several studies demonstrate that ischaemia-reperfusion increases PP2A activity. For example, in rat hippocampus, cerebral ischaemia-reperfusion increases PP2A activity independently of changing protein abundance. This effect is partially reversed by TPCK, a sphingomyelinase inhibitor, implicating ceramide in modulation of PP2A activity (Tian, Qiu, Zhao, Li, & Guo, 2009). PP2A activity and abundance is also increased in cardiac tissue following ischaemia-reperfusion (Kronenbitter et al., 2018; Szobi et al., 2016). Intriguingly, the increase in PP2A activity is associated with dephosphorylation of phospholamban and impaired calcium cycling (Kronenbitter et al., 2018). As a side note, in the study by Szobi, simvastatin prevented the ischaemia-reperfusion mediated increase in PP1β but not PP2A abundance (Szobi et al., 2016). Whether PP2A activity is increased or decreased may depend on the timing when samples are analysed. For instance, 1 hr post-reperfusion following cerebral occlusion, PP2A phosphorylation is decreased, but from 6 h onwards it is increased, as is PP2Ac abundance (Hu et al., 2009). In this study, ischaemia-induced activation of Src caused phosphorylation PP2A at Tyr^{307}, consistent with inhibition of activity as reperfusion progresses.

There is also a body of data demonstrating that ischaemia-reperfusion does not alter PP2A activity or abundance in rat brain or heart (Majd, Power, Koblar, & Grantham, 2016; Morioka et al., 1999; Tobisawa et al., 2017 ; Zhou et al., 2014). In rat heart, ischaemia-reperfusion does not alter PP2A-A, B55α or PP2Ac abundance (Tobisawa et al., 2017; Zhou et al., 2014). Having said that, in a subgroup of animals with chronic kidney disease, ischaemia-reperfusion decreased B55α abundance and increased cardiac infarct size (Tobisawa et al., 2017). Although preconditioning does not alter Ser/Thr phosphatase activity in a rat isolated heart model, pharmacological inhibition of PP2A improves cardiac function when instigated during the reperfusion period (Fan, Van Vuuren, Genade, & Lochner, 2010). It is noteworthy that administration of okadaic acid or cantharidin during the preconditioning phase, decreased recovery, and increased infarct size. In many of the above studies, PP2A abundance/activity is measured during the reperfusion period, this is an important observation because PP2A activity is decreases during the ischaemic phase but recovers following reperfusion in a mouse model of oxygen glucose deprivation (Hedou et al., 2008).

So how does ischaemia *per se* alter the PP2A system? Not surprisingly, there are opposing views. In the rat brain, permanent occlusion of the middle cerebral artery decreases PP2A and PP2Aβ subunit abundance (Koh, 2011, 2012, 2013; Luo et al., 2014; Shah, Park, Gim, & Koh, 2015; Sung et al., 2010). In these studies, melatonin, ferulic acid and curcumin, which have antioxidant or anti-inflammatory properties, prevent the ischaemia-mediated decrease in PP2A abundance (Koh, 2012, 2013; Shah et al., 2015) affording neuroprotection, which further establishes ROS as important mediators of the PP2A system. Similarly, in a rat model of cerebral ischaemia, the decrease in PP2A abundance is restored by oestradiol (Sung et al., 2010). Intriguingly, in a study by Luo et al., acupuncture increased cerebral blood flow, neurogenesis, and partially reversed the effect of ischaemia on PP2A levels (Luo et al., 2014). Regarding the heart, global ischaemia decreases PP2A and PP1 activity in cardiac biopsies and in myocytes. Interestingly, pre-treatment with fostriecin a PP2A inhibitor, reduces infarct size comparable to the protection afforded by preconditioning (Weinbrenner et al., 1998).

In contrast to these studies, ischaemia increases PP2A abundance and B56 subunit expression a rat kidney cell line subjected to ischaemia for 3 h (Tsao et al., 2007). Importantly, silencing of PP2A or exposure to okadaic acid was cyto-protective, while overexpression of B56α, but not B55, was detrimental (Tsao et al., 2007). More recently, in rat hippocampal neurones, oxygen glucose deprivation has also been shown to increase PP2A abundance. This is associated with decreased phosphorylation of ERK1/2, and an increase in ROS, and a depletion of antioxidant/free radical scavenging mechanisms (Yu et al., 2017). Moreover, the increase in PP2A abundance was normalised following exposure to NaHS (sodium hydrogen sulphide) (Yu et al., 2017), most likely due to its antioxidant and free radical scavenging properties (Ahmad et al., 2015).

Based upon the data outlined above, the consensus is that ischaemia-reperfusion and ischaemia, attenuate PP2A activity and abundance through free radical-mediated oxidative damage.

8. PP2A and hypoxia (Table 5)

Hypoxia is defined as an O_2 level of 0.1-1% (Mckeown, 2014) such that normal cellular metabolism and function are compromised. It frequently results from respiratory failure or environmental conditions (high altitude). At a cellular level, hypoxia rapidly and reversibly modulates cell signalling, contractility, ion flux, and redox state. This is critical for maintenance of neural, cardiovascular, and pulmonary function during times of compromised oxidative metabolism. A secondary slower response involves transcriptional suppression, which is mediated by a family of heterodimeric hypoxia-inducible transcription factors (HIF-1-3) (Tian, Mcknight, & Russell, 1997; Wang & Semenza, 1993). Crucially, hypoxia is associated with more that 300 different post-translational modifications including phosphorylation and nitrosylation (Kumar & Klein, 2004). Given the importance of HIF-1α, and post-translational modification in response to hypoxia, what is the evidence for hypoxia-mediated modulation of the PP2A system?

In keeping with HIF-1α mediated suppression of transcription, intermittent hypoxia decreases PP2A mRNA and abundance in a pattern specific manner in rat brain (Raghuraman et al., 2009). The decrease in PP2A abundance appears to be phosphatase specific as PP2C, PP4, PP5 and PP6 are unaffected, at least by intermittent hypoxia (Raghuraman et al., 2009). Importantly, in a porcine model of global hypoxia, total protein phosphatase and PP2Ac activity are decreased in nuclear and membrane fractions, but not cytosolic preparations isolated from cerebral cortex (Truttmann, Ashraf, Mishra, & Delivoria-Papadopoulos, 2004). The loss of activity likely reflects increased phosphorylation and demethylation of PP2Ac, as occurs following prolonged intermittent mild hypoxia (10% O_2, 6 h/day for 1-8weeks) in the rat hippocampus (Zhang et al., 2014) and rosette leaves of Arabidopsis (Creighton et al., 2017). While the kinase responsible for phosphorylation of PP2A remains to be determined, ROS are likely involved. This is based upon data showing that: 1) loss of PP2A activity is associated with an increase in malondialdehyde levels (Zhang et al., 2014), a marker of lipid peroxidation, and 2) antioxidants prevent hypoxia-mediated attenuation of PP2A abundance (Raghuraman et al., 2009).

Several groups have also shown hypoxia to upregulate PP2A. In human colorectal cancer cells, rat primary alveolar epithelial cells and mouse heart, hypoxia increases PP2A activity (Caraballo et al., 2011; Larsen et al., 2008; Lin, Lee, et al., 2012), PP2A mRNA and protein abundance (Chen et al., 2014; Lu et al., 2017; Zhu et al., 2015). While hyperoxia (95% O_2, 1-24 h) decreases PPA activity, independently of altering PP2A abundance in macrophages (Nyunoya, Monick, Powers, Yarovinsky, & Hunninghake, 2005). Although the increase in hypoxia-mediated PP2A activity could be explained by the increase in PP2A abundance, it more likely reflects post-translational modification of PP2Ac. For instance, in HT29 human colorectal cancer cells exposed to hypoxia, the increase in PP2A activity was accompanied by decreased phosphorylation of PP2Ac, independently of altering levels of PP2A or PP2A Aα/β (Lin, Lee, et al., 2012). Again, several studies indicate that ROS may be responsible for the increase in PP2A abundance (Lu et al., 2017; Zhang et al., 2014; Zhu et al., 2015), as the effect is attenuated on exposure to ROS scavengers (1-10-phenanthroline and superoxide dismutase) (Chen et al., 2014).

In contrast to the above studies, a couple of groups demonstrate that hypoxia does not in fact alter PP2A expression or abundance (Hofstetter et al., 2012; Kucera et al., 2017). Whether co-incidental or not, these studies were undertaken in tumour derived stem cells and mouse embryonic stem cells, respectively.

9. Is there a link between PP2A and HIF-1α?

One of the first studies to establish a link between HIF-1α and PP2A was by Komatus et al. in 2007. In this study, PP2A and PP2Acβ mRNA was found to be lower in fractures of the femur from HIF-$1\alpha^{+/-}$ mice than those with a HIF-$1\alpha^{+/+}$ phenotype (Komatsu, Bosch-Marce, Semenza, & Hadjiargyrou, 2007). In a more recent clinical study, PP2A activity was shown to correlate with HIF-1α abundance in patients with a high-grade astrocytoma compared to non-neoplastic surgical specimens, and was an independent predictor of survival (Hofstetter et al., 2012). This is consistent with the observation that knockdown of HIF-1α increases proliferation of ovarian cancer cells by reducing PP2A activity, but not PP2Ac abundance in an ovarian clear cell carcinoma cell line (Takai et al., 2015). Recently, work by our group has shown that in HAEC, HASMC, HCF-av and AC16 cells expression of multiple components of the protein phosphatase system are suppressed by hypoxia with kinetics that are cell line dependent. When investigated further in HASMC and AC16 cardiomyocytes, PP2A was found to be attenuated through both a HIF1α dependent and independent mechanism. The latter is consistent with altered PP2A holoenzyme assembly (Elgenaidi and Spiers, 2019).

Interestingly, the evidence is not limited to HIF-1α *per se*, as HIF-prolyl-hydroxylase 2 (PHD2), which mediates degradation of HIF-1α, can hydroxylate B55α at Pro^{319} leading to its degradation (Di Conza, Trusso Cafarello, Zheng, Zhang, & Mazzone, 2017). There is also a reciprocal interaction, as B55α-associated PP2A attenuates degradation of HIF-1α through dephosphorylation of PHD2 at Ser^{125} to increase HIF-1α levels (Di Conza, Trusso Cafarello, Zheng, et al., 2017). Again, ROS may underpin HIF-1α mediated modulation of PP2A, as ROS can activate or inhibit the HIF-1α signalling pathway (Movafagh, Crook, & Vo, 2015).

10. Pharmacological targeting of the PP2A system: therapeutic potential?

It is evident that oxidative stress plays a fundamental role in modulating the PP2A system. Therefore, it is surprising there is a paucity of data regarding pharmacological modulation of the PP2A system in response to oxidative stress. This is a significant omission given the importance of protein phosphorylation in cell signalling, and that approximately 75% of phosphosites are serine and threonine residues; 55-70% of which are dephosphorylated by a PP2A holoenzyme (Eichhorn et al., 2009; Kauko et al., 2018). Nevertheless, there is information on pharmacological manipulation of the PP2A system relating primarily to cancer, which is worth considering as hypoxia and HIFs are relevant to tumourigenesis (Samanta & Semenza, 2018).

10.1. Direct targeting of PP2A: SMAPs and LB-100

The ground work for the development of small molecule activators of PP2A (SMAPs) stemmed from a screen of FDA approved drugs, which found that certain phenothiazines induced PP2A mediated apoptosis in lymphoblastic leukaemia (Gutierrez et al., 2014). It was in the process of decoupling the CNS pharmacology of the phenothiazines from their antiproliferative effects that SMAPs were born. In a seminal paper by Sangodkar, SMAPs were shown to inhibit growth of KRAS mutant lung cancer cells by decreasing pERK-mediated signalling (Sangodkar et al., 2017). Importantly, they showed SMAPs bind to HEAT repeats 5-8 of the PP2A-Aα subunit (Fig. 5), and that mutation of Lys^{194}, Glu^{197} and Leu^{198} conferred drug resistance. Although the B subunit binding site overlaps this site, B subunit binding does not alter SMAP binding, at least for holoenzymes composed of the B56 subunit. Importantly, the authors went on to show that binding of SMAPs to PP2A-Aα induces a conformational change in PP2A, which increases its activity (Sangodkar et al., 2017).

In A539 and H258 cells exposed to the SMAP, TRC-794 (aka DT-794; Fig. 6), phosphoproteomic analysis showed that while different signalling pathways were modulated in each cell line, there was commonality regarding down regulation of RAS and cell cycle kinase signalling (Wiredja et al., 2017). Phosphoproteomic analysis of castrate resistant prostate cancer cells has demonstrated that SMAPs-induced cytotoxicity is associated with dephosphorylation of the androgen receptor and down-regulation of its associated kinases (Mcclinch et al., 2018). This adds to an earlier study demonstrating that penfluridol (antipsychotic) which has a diphenylbutylpiperidine backbone, elicits an anti-tumour effect through PP2A mediated dephosphorylation of Akt, p70S6K and GSK3β in pancreatic cancer cells (Chien et al., 2015)

There are also several reports regarding LB-100 (Fig. 6), which is a small molecule inhibitor of PP2A (Fig. 5). In one study, LB-100 enhanced cisplatin-mediated cytotoxicity in an *in vitro* model of medulloblastoma, through dephosphorylation of STAT3 and several of its down downstream targets (Ho et al., 2016). In the same study, LB100 abrogated cisplatin-induced drug resistance by increasing cisplatin uptake in cells. Many of the other studies regarding LB-100, also demonstrate chemosensitisation when given in combination with tyrosine kinase inhibitors (Lai et al., 2018), and daunorubicin (Hu et al., 2017). In a similar vein, LB-100 sensitises several cancer cell lines to radiation therapy (Hao et al., 2018; Ho et al., 2018).

In addition to the above, low dose microcystin-LR has been shown to increase PP2A activity in HEK293 cells by promoting dissociation of α4 from PP2Ac (Li et al., 2011). However, at high concentrations PP2A is inhibited (Mackintosh, Beattie, Klumpp, Cohen, & Codd, 1990).

10.2. Targeting the endogenous inhibitors

10.2.1. SET antagonism

Pharmacological targeting of SET centres around ceramide and FTY-720 (fingolimod; Fig. 6). In early work involving rat T9 glioma cells, Dobrowsky found that ceramide (N-acylsphingosine) but not related sphingolipids activated the heterotrimeric PP2A holoenzyme (PP2A-ABC) but not PP2A-AC or PP2Ac (Dobrowsky, Kamibayashi, Mumby, & Hannun, 1993). This was subsequently verified, and extended to show C_{18} ceramide activates PP2Ac, PP1γc and PP1αc (Chalfant et al., 1999). However, it wasn't until 10 years later that SET (I_2^{PP2A}) was identified as the ceramide binding protein (Fig. 5), exhibiting stereoisomer specificity and fatty acid chain length preferences (Mukhopadhyay et al., 2009). In this study, the authors established that binding of ceramide to a trimer of amino acids (Val^{207}, Ile^{208} and Lys^{209}) on helix 7 of the

Table 5
Effect of hypoxia on PP2A phosphatase

Cell line/Tissue	Model	Protocol	PP2A Response	Notes	Additional Information	Reference
Rat Brain stem	Intermittent hypoxia	5% O_2 15s: 21% O_2 5min	↓ PP2A mRNA & abundance	↑ ROS; Antioxidants prevent ↓ PP2A; ↔ PP2C, PP4, PP5, PP6	10% O_2:21% O_2 in 9 s cycles ↔ effect	(Raghuraman et al., 2009)
New born piglets	Hypoxia	FiO_2: 0.07-0.08 for 1 h to give P_aO_2 <25 mmHg	↓ Total PP & PP2A activity in nuclei and membrane; ↔ PP2A activity in cytosol	↑ CaMK IV	PP2A modulates programmed cell death via CaMK IV	(Truttmann et al., 2004)
HT29 human colorectal cancer	Hypoxia	1% O_2, 94% N_2, 5% CO_2	↑ PP2A activity, ↓ pPP2A abundance in $CD133^+$ vs $CD133^-$ cells; ↔ PP2A abundance or PP2A-Aα/β	↓ pP38 MAPK		(Lin, Lee, et al., 2012)
Arabidopsis	Root Hypoxia	1 week submerged in Hoagland solution	↑ demethylated PP2Ac and PP2Ac abundance	In rosette leaves		(Creighton et al., 2017)
PC12 Cells	Hypoxia intermittent	5% O_2, 30 min cycles	↑ PP2A mRNA and abundance	1, 10-Phenanthroline, SOD prevented ↑ PP2A ↓ ERK1/2 prevented by OA, cantharidin, SOD	ROS, PP2A, ERK1/2 decrease cell proliferation	(Chen et al., 2014)
Rat Brain, hippocampus	Hypoxia	10% O_2 for 6 h/day over 4 weeks	↑ pPP2CA (1-8 weeks); ↓ mPP2CA (4 & 8 weeks)	↑ pGSK3β (Tyr^{216}) ↑ malondialdehyde ↔ SOD		(Zhang et al., 2014)
HUVEC	Hypoxia reoxygenation	1% O_2 for 1 h, reoxygenation 2 h	↑ PP2A abundance	↑ ICAM, E-selectin & O_2^-, ↓ phospho-eNOS & NO	Protective effect of propofol mimicked by calyculin A; antagonised by FTY-720	(Zhu et al., 2015)
HT22 cells (Mouse)	Hypoxia	$CoCl_2$ 12 h	↑ PP2A abundance	↑ phospho-$nNOS^{1412}$ ↓ phospho-$nNOS^{847}$ Protective effect of propofol mimicked by OA; antagonised by FTY-720	Propofol protective via PP2A, nNOS pathway	(Lu et al., 2017)
Rat primary alveolar epithelial cells	Hypoxia	PO_2 50 mmHg for 1 h	↑ PP2A activity	↓ occludin ↔ E-cadherin or claudin OA prevents ↓ occludin	↓ Permeability via PP2A pathway	(Caraballo et al., 2011)
Mouse LV	Alveolar hypoxia	10% O_2 for 1-14 days	↑ PP2A abundance and activity in SR enriched membranes	↔ PP1 activity	↑ PP2A dephosphorylates phospholamban at Ser^{16}	(Larsen et al., 2008)
Tumour derived stem cells	Hypoxia	$CoCl_2$ 2-24 h 1% O_2 for 6 h	↑ PP2A activity; ↔ PP2A abundance	PP2A mediated growth inhibition OA ↑ proliferation PP2A shRNA ↑ proliferation	PP2A activity ∝ HIF-1α abundance in glioblastoma.PP2A activity predictor of poor survival	(Hofstetter et al., 2012)
Mouse embryonic stem cells	Hypoxia	1% O_2 for 24 h	↔ PP2A mRNA	Dephosphorylation of ERK and upstream kinases	DUSPs and PP2A limited role in hypoxia mediated attenuation of ERK signalling	(Kucera et al., 2017)
RAW 264.7 macrophages	Hyperoxia	95% O_2 for 1-24 h	↓ PP2A activity; ↔ PP2A abundance	↑ ERK activation ↓ MAPK3 activity ↓ MAPK3 abundance	Hyperoxia promotes survival via ERK activation due to ↓ phosphatase activity	(Nyunoya et al., 2005)

Abbreviations: CAMK IV, calcium calmodulin kinase IV; DUSP, dual specificity phosphatase; eNOS, endothelial nitric oxide; ERK, extracellular signal related kinase; GSK3β, glycogen synthase kinase 3β; HIF-1α, hypoxia inducible factor 1α; ICAM, intercellular adhesion molecule; MAPK, mitogen activated protein kinase; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; OA, okadaic acid; shRNA, short hairpin ribonucleic acid; SOD, superoxide dismutase.

SET protein disrupts the interaction between SET and PP2A, to disinhibit PP2A.

FTY-720 is another sphingosine analogue, derived from the fungal metabolite myriocin, licensed for the treatment of multiple sclerosis. FTY-720 is an immunomodulator with cytotoxic properties, which has led to investigation of its use in cancer. In leukaemia cells, the cytotoxic effect of FTY-720 is mediated through activation of PP2A (Fig. 5), and dephosphorylation of ERK and AKT (Matsuoka, Nagahara, Ikekita, & Shinomiya, 2003; Smith et al., 2016), key oncogenic proteins (Janssens & Goris, 2001; Mumby, 2007). Although FTY-720 can undergo phosphorylation, its cytotoxicity is not dependent on this, as non phosphorylatable chiral deoxy analogues of FTY-720 activate PP2A in experimental models of AML and asthma (Collison et al., 2013; Toop et al., 2016). However, binding of small T antigen to the PP2A dimer (AC subunits) or free PP2Ac abrogates the proapoptotic effect of FTY-720 by interrupting holoenzyme formation (Neviani et al., 2007). This has been refined to show that FTY-720 activates PP2A through down regulation of SET and dephosphorylation of PP2Ac (Yang, Huang, Lu, Li, & Huang, 2012). Nevertheless, activation of the PP2A holoenzyme by FTY-720 is likely more complex as it also upregulates scaffolding and several regulatory subunits including B55α of PP2A (Ruvolo et al., 2014; Shouse, De Necochea-Campion, Mirshahidi, Liu, & Chen, 2016; Yang et al., 2012).

ApoE and ApoE peptide mimetics form another group of SET antagonists (Fig. 5). When investigating the immunomodulatory properties of ApoE and its mimetics, Christensen et al. discovered that COG112 and COG449 (renamed OP449; Fig. 6) bind to the C-terminal region of SET to enhance PP2A activity (Christensen, Ohkubo, et al., 2011). The PP2A activation by ApoE mimetics has been confirmed in subsequent studies, and their utility in cancer models established (Agarwal et al., 2014; D'souza et al., 2016). However, ApoE has recently been reported to decrease PP2A activity through: 1) transcriptional repression of PPP2R5E (B56ε) and an associated reduction in B56ε protein abundance, and 2) triggering demethylation of PP2Ac (Theendakara et al., 2017). Both of which are consistent with inhibition of PP2A activity. This is not surprising as ApoE is a recognised transcription factor for ~3000 genes (Theendakara et al., 2016). Finally, TGI1002 (Fig. 6), a novel 2-phenyloxypyrimidine based on an imatinib backbone, also acts as a SET antagonist. By binding to SET, TGI1002 disrupts the interaction between SET and PP2A, the consequence of which is an increase in PP2A activity (Wang et al., 2015).

10.2.2. CIP2A Antagonism

Mechanistic insight into the anti-tumour effects of the proteasomal inhibitor bortezomib and the receptor tyrosine kinase inhibitor erlotinib (Fig. 6), has revealed they can function as CIP2A 'antagonists' (Fig. 5). For example, in hepatocellular carcinoma, colon cancer, leukaemia, breast cancer, head and neck squamous cell carcinoma cell lines, the anti-tumour/pro apoptotic effects of bortezomib are mediated through transcriptional downregulation of CIP2A resulting in dephosphorylation of Akt as a consequence of enhanced PPA activity (Ding et al., 2014; Lin, Chen, Chen, Cheng, & Chen, 2012; Liu, Shiau, et al., 2013; Tseng et al., 2012b; Yu, Hou, et al., 2013). Importantly, the latter was not due to altered PP2A abundance (Lin, Chen, et al., 2012) or proteasomal degradation (Tseng et al., 2012b). But appears to involve down regulation of the transcription factor p4EBP-1 (Yu, Hou, et al., 2013). Moreover, the CIP2A-mediated effects were only observed in cell lines responsive to bortezomib (Chen et al., 2010; Liu, Shiau, et al., 2013; Tseng et al., 2012b). Similarly, several novel bortezomib derivatives including ΔBTZ (Fig. 6) which 'lack' a proteasomal action have similar effects on CIP2A, dephosphorylation of Akt, and cell proliferation (Hou et al., 2013; Yu, Hou, et al., 2013). These observations are strengthened by data demonstrating silencing of PP2A or ectopic expression of CIP2A to prevent bortezomib-mediated apoptosis and dephosphorylation of Akt (Chen, Yu, et al., 2011; Lin, Chen, et al., 2012).

Similarly, in non-small cell carcinoma and hepatocellular carcinoma cells, erlotinib (Fig. 6) down-regulates CIP2A and increased PP2A activity (Wang et al., 2014; Yu, Chen, et al., 2013). Importantly, the apoptotic effect of erlotinib was reversed by ectopic expression of CIP2A (Wang et al., 2014; Yu, Chen, et al., 2013). In subsequent studies, TD-52 and TD-19 (Fig. 6), which are based upon the erlotinib backbone, have been shown to reactivate PP2A via transcriptional repression of CIP2A in hepatocellular carcinoma cells (Chao et al., 2014;Liu et al., 2017 ; Yu et al., 2014) as they prevent the binding of Elk-1 to the CIP2A promoter (Liu et al., 2017; Yu et al., 2014). Disubstituted quinazoline and pyrimidine derivatives of erlotinib also exhibit antiproliferative effects and inhibit CIP2A and pAkt expression (Chen et al., 2012). Surprisingly, forskolin (Fig. 6), which is a well-established tool to activate adenylate cyclase, sensitises drug resistant HA59T cells to erlotinib through activation of PP2A (Yu, Chen, et al., 2013).

Celastrol (Fig. 6), a pentacyclic triterpenoid isolated from *Tripterygium Wilfordii* Hook F is another CIP2A "antagonist" (Fig. 5). In non-small cell carcinoma, celastrol stimulates proteasomal degradation of CIP2A via CHIP (carboxy terminus of HISP70 interacting protein) E3 ligase (Liu et al., 2014). The consequence of which is suppression of CIP2A-Akt signalling and potentiation of cisplatin-mediated cytotoxicity (Liu et al., 2014). In support of these findings, a more recent study demonstrats that overexpression of CIP2A prevents celastrol mediated cytotoxicity and reverse its transcriptional repression of CIP2A and cMyc (Wu et al., 2017). Finally, rabdocoetsin B (Fig. 6), a diterpenoid extracted from *Rabdosia coetsa* also downregulates CIP2A and decreases cell proliferation in lung cancer (Ma et al., 2011)

10.3. PME and LCMT-1

Very few small molecule inhibitors of PME-1 or LCMT-1 have been identified. However, a high-throughput screen using fluorescence polarization-activity-based protein profiling (FluoPol-ABPP) identified aza-β-lactams and sulphonyl acrylonitrile-based compounds as PME-1 antagonists (Fig. 5). Of these, ML174 and AMZ30 (Fig. 6) were the most potent and selective inhibitors with regard to demethylation of PP2A in cells (Bachovchin et al., 2010; Bachovchin et al., 2011). Lee et al. showed that eicosanoyl-5-hydroxytryptamide (Fig. 6) is another possible PME-1 inhibitor, as it prevents demethylation of PP2A-AC dimers in an *in vitro* assay and in cultured neurones (Lee et al., 2011). Interestingly, while AMZ30 and ABL127 have anti-proliferative properties in an *in vitro* model of endometrial carcinoma, eicosanoyl-5-hydroxytryptamide had no effect. Surprisingly, none of the compounds

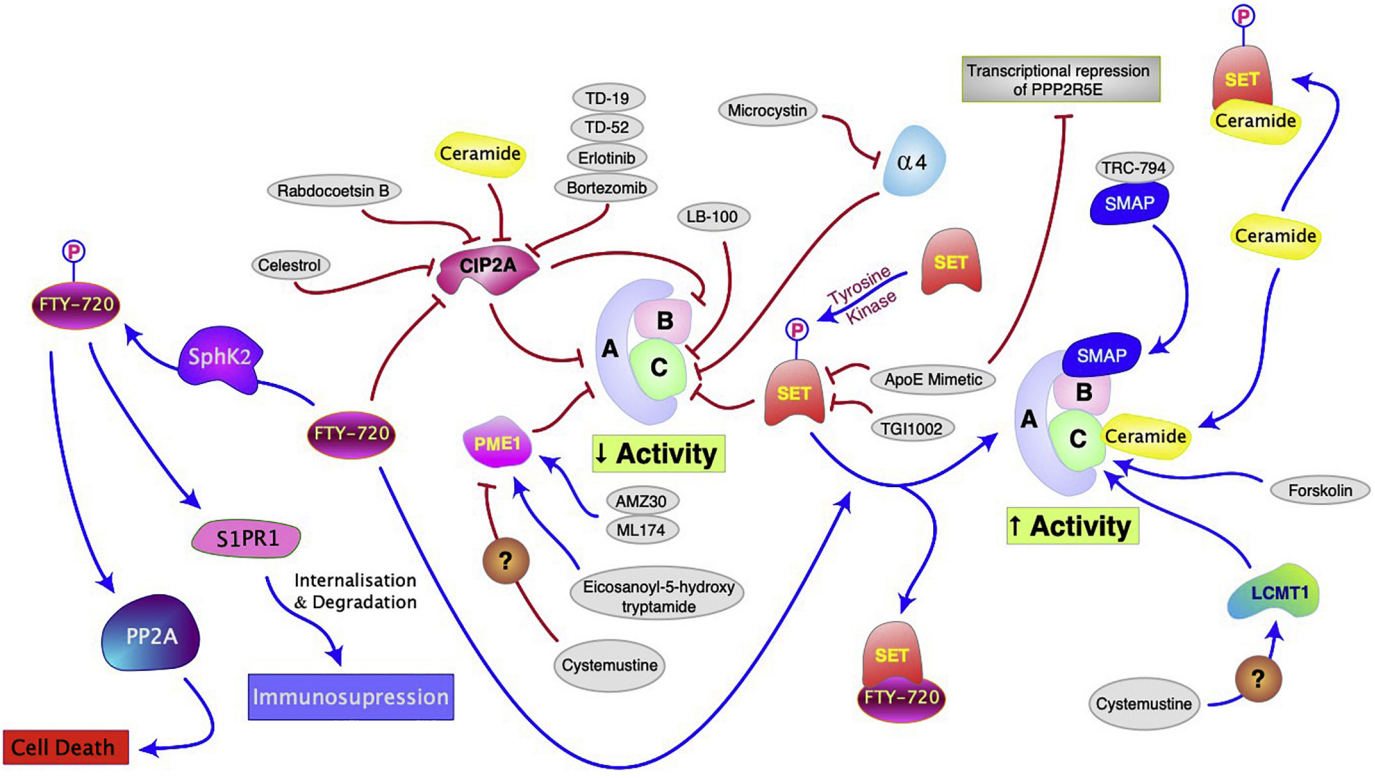

Fig. 5. A schematic diagram illustrating the site of action of pharmacological agents targeting the PP2A system. The activity of PP2A can be enhanced using CIP2A antagonists (FTY-720, erlotinib, bortezomib), SET antagonists (TGI1002, ApoE mimetics) and through inhibition of PME-1 (Aza-β lactams). Alternatively, PP2A can be activated 'directly' by small molecule inhibitors of PP2A (SMAPs) and ceramide. *Abbreviations:* CIP2A, cancerous inhibitor of PP2A; LCMT-1, leucine carboxyl methyltransferase-1; SMAP, small molecule activator of PP2A; SphK2, sphingosine kinase 2; S1PR1, sphingosine-1-phosphate receptor 1.

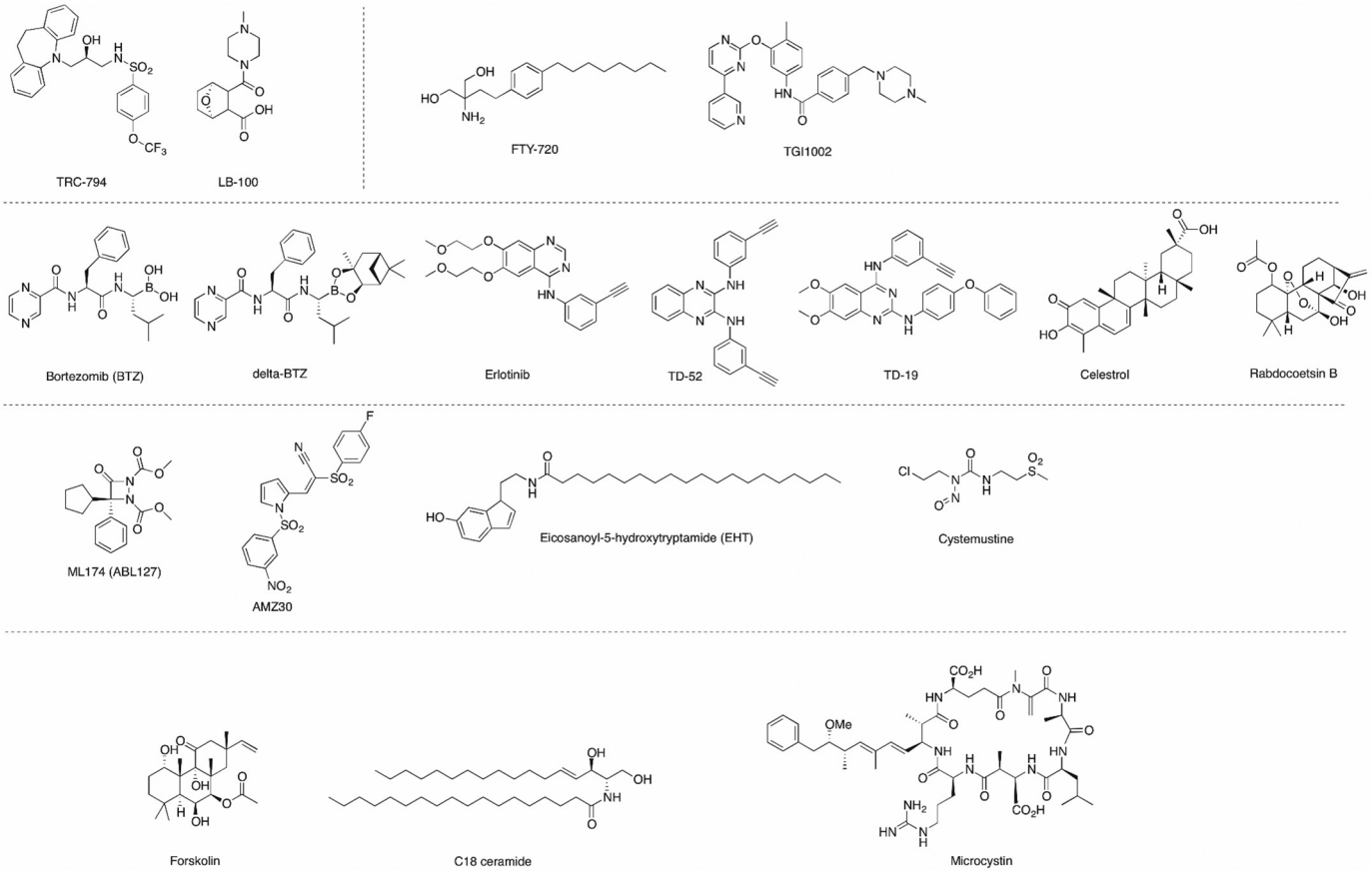

Fig. 6. Chemical structures of novel and established pharmacological agents targeting the PP2A system

had an anti-tumour effect in a xenograft model at the concentrations used (Pusey et al., 2016).

Juxtaposed to the above, the anti-tumour properties of cystemustine (Fig. 6), a chloroethyl nitrosourea, is accounted for in part by increased methylation of PP2A, and decreased activation of Akt and cMyc expression (Guenin et al., 2008). However, it remains to be determined if this is due to inhibition of PME-1, activation of LCMT-1 or simply a response to cellular damage.

11. Future direction

Although there is a significant body of evidence to support oxidative stress-mediated regulation of the PP2A system, many of the studies are conflicting and primarily focused on PP2Ac. In this regard, a better understanding of how oxidative stress regulates PP2A is required to clarify the disparity. Given the importance of the regulatory and scaffolding subunits in determining localisation and substrate specificity of the PP2A holoenzyme, work in this area is required to fill the void. A better understanding of this is critical if the utility of small molecule inhibitors of PP2A, and PP2A are to be exploited therapeutically. The role of PP2A, as a key modulator of the oxidative stress response is a developing field, ripe for rich pickings to those in the area or wishing to dip their toe in.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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