Modulation of serine/threonine-protein phosphatase 1 (PP1) complexes: a promising approach in cancer treatment

Modulation of Protein phosphatase 1 complexes: a promising approach in cancer treatment

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Teaser: The modulation of PP1 complexes by small molecules or peptides offers a promising strategy to delay the progression of cancer, highlighting their potential application as anti-cancer therapies.

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Abstract

Cancer is the second leading cause of death worldwide. Despite the numerous therapeutic options available, tumor heterogeneity and chemoresistance have limited their success and the development of an effective anticancer therapy remains a major challenge in oncology research. The serine/threonine-protein phosphatase 1 (PP1) and its complexes have been recognized as potential drug targets. Although research on the modulation of PP1 complexes is currently at an early stage, there is an immense potential. Chemically diverse compounds have been developed to disrupt or stabilize different PP1 complexes in various cancer types with the objective to inhibit disease progression. Beneficial results obtained *in vitro* now require further pre-clinical and clinical validation. In conclusion, the modulation of PP1 complexes seems to be a promising, albeit challenging, therapeutic strategy for cancer.

Keywords: PP1 complexes, cancer treatment, small molecules, peptides

Introduction

According to the World Health Organization (WHO), cancer is considered a major public health concern, estimated to be the second leading cause of death worldwide. The incidence of cancer has latterly increased with a total of 18.1 million new cases and 9.6 million deaths reported globally in 2018.[1] Currently, therapy decision is dictated by cancer type and clinical staging. Options include both localized therapies, including surgery or radiation therapy, and systemic therapies which encompass chemotherapy and hormonal and immune interventions. The success of conventional therapies is essentially limited by tumor heterogeneity and their acquired resistance to therapy.[2] More detailed knowledge of tumors' molecular biology has, in the past decade, provided important advances coupled with the emergence of new approaches for cancer treatment, including more personalized cancer medicine. Nevertheless, serious challenges remain and the establishment of improved therapies is urgently needed.[2]

Targeting the post-translational phosphorylation of intracellular proteins has been considered a viable anticancer therapy. Transient phosphorylation events control most cellular signaling processes, whereas abnormal phosphorylation profiles have been associated with several pathological conditions, including cancer.[3] The phosphoproteome is the consequence of the activities of both protein kinases and phosphatases, which add or remove phosphate groups respectively. The balance between these two types of enzyme is essential to maintain cellular homeostasis.[4] Thus, targeting both protein kinases and phosphatases has been proposed for cancer treatment.[5,6]

The serine/threonine-protein phosphatase 1 (PP1) is a major protein phosphatase, catalysing a wide range of protein dephosphorylation reactions in human cells.[7] It regulates critical cellular processes including cell cycle progression, apoptosis and metabolism.[8] The involvement of PP1 in several oncogenic pathways has becoming evident and its expression levels seems to be altered in the presence of a tumor.[9] Nevertheless, the direction of the alteration in PP1 expression levels is not clear, since contradictory results have been published. Importantly, PP1 deregulation seems to depend on the type of cancer, on the interacting proteins as well as on the PP1 isoform.[10–13] Indeed, the catalytic subunit of PP1 (PP1c) is encoded by three genes – *PPP1CA*, *PPP1CB*, *PPP1CC*, giving rise to three different isoforms (PP1-alpha catalytic subunit (PP1α), PP1-beta catalytic subunit (PP1β) and PP1-

gamma catalytic subunit (PP1Ɣ)) that are ubiquitously expressed and differ mainly in their extremities.[14] The roles of PP1 depends on the interaction of PP1c with different regulatory interactors of PP1 (RIPPOs)[15] (previously called PP1-interacting proteins (PIPs)), which can act as targeting subunits, substrates, activity regulators, or both. A determined effort over several decades has identified the PP1c interactome in different tissues and specific biological contexts, including pathological conditions.[16–19] Despite the relatively high number of PP1 complexes identified in human tissues, the highly dynamic nature of these complexes has clearly hampered their functional characterization.[20]

Targeting PP1 has been considered for the treatment of several other diseases, including heart failure[21] and neurological conditions.[22] Compared with conventional chemotherapies, the modulation of discrete PP1 complexes could provide a more specific option with reduced cytotoxicity. In fact, this novel approach has been proposed for the treatment of various pathologies,[23–25] including cancer.

In this context, we rigorously reviewed the potential of modulating PP1 complexes in cancer treatment. Herein, we summarize the PP1 complexes characterized in different types of cancer, highlighting their roles as tumor promoters or suppressors. The PP1 complexes modulated by either small molecules or peptides in cancer are also described. Finally, we define the main conclusions that can be drawn from the studies and the principal challenges of the future work in this topic.

PP1 complexes in cancer: tumor promoters or suppressors?

The interaction of PP1c with its regulatory interactors plays important roles in key oncogenic pathways. Furthermore, dysregulation of some PP1 complexes has been associated with cancer initiation and/or progression.[26] Contradictory roles have been attributed to different PP1 complexes in cancer. Indeed, some are considered tumor promoters, while others are associated with a tumor suppressor activity. The tumor promoter/ suppressor activity of PP1 holoenzymes seems to principally depend upon the influence of different RIPPOs on PP1c activity. The cellular consequences of PP1c-mediated dephosphorylation are further complicated by the fact that both oncogenes and tumor suppressor proteins are known substrates and that such events can activate or inhibit these target proteins.

In this section we have revised the major PP1 complexes characterized in different types of cancer with identified roles as tumor promoters or suppressors; these findings are summarized in **Table 1** and schematized in **Figure 1**.

Breast cancer

Several PP1 complexes have been identified in breast cancer (BCa) cell lines and *in vivo* animal models, being already reported important roles in promoting tumor progression. The transcriptional coactivator with PDZ-binding motif (TAZ) interacts with and is dephosphorylated by PP1α, leading to TAZ activation.[27] TAZ is a transcriptional co-activator downstream of the Hippo pathway, involved in several cellular processes including cell proliferation and epithelial-mesenchymal transition.[28] By activating TAZ, the TAZ/PP1c complex was associated with BCa cell proliferation.[27] The breast cancer type 1 susceptibility protein (BRCA1) is also a substrate of PP1c and its dephosphorylation, contrary to TAZ, negatively affects its function. BRCA1/PP1c complexes also inactivate several BRCA1-related proteins, by targeting PP1c to dephosphorylate them. The inhibition of the tumor suppressor activity of these proteins is associated with the development of BCa.[12] Moreover, serine/threonine-protein phosphatase 1 regulatory subunit 10 (PNUTS)/PP1c complexes seem to affect the binding and consequent dephosphorylation of different PP1 substrates. Indeed, PNUTS/PP1c activated PP1c-mediated dephosphorylation of the Myc proto-oncogene protein (MYC), yet inhibited the PP1c activity against retinoblastoma protein (RB).[29,30] The dephosphorylation of MYC inhibited its degradation, contributing to BCa cells proliferation.[29] On the other hand, the increased levels of phospho-RB inactivated RB tumor suppressor activity, thus contributing to decreased BCa cell apoptosis.[30] Other PP1 complexes were associated with inhibition of PP1c activity against specific substrates, also contributing to BCa initiation/ progression. For instance, the tyrosine-protein kinase Fer (FER) also inhibited the enzymatic activity of PP1c against RB, which contributed to BCa cell cycle progression.[31] The histone deacetylase 6 (HDAC6)/PP1c complex inhibited the activity of PP1c against a specific substrate – protein kinase B (AKT).[32] AKT is activated by phosphorylation, contributing to tumorigenesis.[32,33]

In contrast, various PP1 complexes seem to have a tumor suppressor role in BCa. Some PP1 complexes appear to counteract the effect of other previously mentioned. For instance, phosphatase and actin regulator 4 (PHACTR4)/PP1c complex, contrary to PNUTS/PP1c and

FER/PP1c, induced PP1c activity against RB, activating its tumor suppressor activities, which led to decreased BCa cell proliferation.[34] In addition, protein phosphatase 1 regulatory subunit 7 (SDS22)/PP1c induced PP1c-mediated AKT dephosphorylation, contrary to the effect of HDAC6/PP1c complex. This effect resulted in BCa cell apoptosis.[35] On the other hand, the interaction of the tumor suppressor spinophilin (SPN) with PP1c inhibited the PP1c activity against a subset of substrates by blocking a binding-site of PP1c that is common to various substrates. This resulted in decreased tumorigenic proprieties of BCa cells.[36] The PP1c-mediated dephosphorylation of CREB was also inhibited by a PP1 complex: phospho-protein phosphatase 1 regulatory subunit 1B (p-DARPP-32)/PP1c. This resulted in increased activity of CREB, which was associated with oncogenic potential due to its central position downstream of many growth signaling pathways.[37,38] The tensin1 (TNS1) protein has been considered a regulatory subunit of PP1α, controlling its localization and activity to adhesions, resulting in suppression of BCa cell migration and invasion. A role of TNS1 as a substrate of PP1c was also described and seems to contribute to the complex tumor suppressor activity.[39,40] Furthermore, focal adhesion kinase (FAK) and steroid receptor coactivator 3 (SRC-3) are both dephosphorylated by PP1c, a process which subsequently inactivates their oncogenic activity.[41,42] Both these proteins contribute to tumor progression but have different roles. While FAK was associated with tumor invasion and migration, SRC-3 was considered an oncogene and growth coactivator.[43,44] Finally, some PP1 complexes seem to be formed in response to BCa treatment and seems to contribute to a positive outcome.[45,46] Indeed, caveolae-associated protein 3 (CAVIN3)/PP1c inhibited the PP1c activity, increasing the H2AX phosphorylation and resulting in BCa cells apoptosis in response to UV treatment.[45] The tumor suppressor proapoptotic WT1 regulator (PAR4) interacted with PP1c in response to chemotherapy and altered the phosphorylation of several cytosolic proteins, re-sensitizing the tumors to chemotherapy.[46]

Cervical cancer

Tumor promoter activity has been associated with different PP1 complexes in cervical cancer. The interaction of proliferation marker protein Ki-67 and Repo-Man with PP1γ regulates its activity, mediating PP1γ--specific processes which include histone dephosphorylation and chromatin remodeling.[47] A role in recruiting PP1γ to chromatin, promoting cancer cell survival was also proposed for the Repo-man/PP1γ complex.[48] Both complexes were proposed as

cancer therapeutic targets.[47] The LIM and senescent cell antigen-like-containing domain protein 1 (PINCH1) also interacts with PP1c and inhibits its activity against the specific substrate AKT. This promotes the phosphorylation and consequent activity of AKT, resulting in cancer cell survival and increased resistance to radiation therapy.[49] Furthermore, IKK interaction with PP1c was associated with IKK activation and consequent phosphorylation-mediated degradation of the NF-kB inhibitor IkBα. This PP1 complex enhanced NF-kB-mediated tumorigenesis by the upregulation of genes which promote cancer cell survival and growth. The effect of IKK/PP1c complexes upon PP1c activity is not well understood.[50] The growth arrest and DNA damage-inducible protein (GADD34)/ PP1c complex seems to also contribute to tumor progression by inhibiting the cervical cancer cells apoptosis. This complex promotes the dephosphorylation of eukaryotic translation initiation factor 2A (eIF2α), thus regulating the calreticulin exposure.[51]

Conversely, aurora kinase A (STK15)/PP1c and protein phosphatase 1 regulatory subunit 1C (IPP5)/PP1c seem to inhibit the tumor progression. STK15 is overexpressed in various cancers and associated with oncogenic transformation. Its isomer, aurora kinase B (STK12) is also overexpressed in different types of cancer, including cervical cancer and was associated with tumor invasiveness.[52] Interaction with PP1c dephosphorylates STK15 and STK12, inhibiting their function, which suggest a tumor suppressor activity. PP1c activity is also supressed by STK15-mediated phosphorylation.[53,54] Moreover, IPP5 also inhibits PP1c activity and was related to cervical tumor growth suppression. Nevertheless, the association of this effect with the IPP5 interaction with PP1c is still not clear.[55]

The effect of the nuclear inhibitor of protein phosphatase 1 (NIPP1)/PP1c complex in cervical cancer is controversial. It is consensual that NIPP1 inhibits the activity of PP1c but while inhibition of tumor growth was reported by some authors,[56] others described a contribution to migratory proprieties.[57] We hypothesize that the interaction with different PP1c isoforms may, at least in part, justify these contradictory results.

Colorectal cancer

Similarly to what was described for other types of cancer, several PP1 complexes contributing to tumor progression were identified in colorectal cancer. INH3 and caveolin-1 (CAV1) bind to and inhibit PP1c activity. The INH3/PP1c complex mediates the INH3 activating effect

against signal transducer and activator of transcription 3 (STAT3), which is involved in metastasis formation.[58,59] A role in colorectal cancer cell migration and invasion, by increasing phospho-AKT and kallikrein-6 (KLK6) secretion, was described for the CAV1/PP1c complex, which also contributes to metastasis.[60] Both PNUTS/PP1c and PINCH1/PP1c are functional in colorectal cancer by inhibiting the activity of PP1c against specific substrates, as described for breast and cervical cancers respectively.[30,49] Lastly, the Yes-associated protein 2 (YAP2) is considered a substrate of PP1c and is stabilized by dephosphorylation, resulting in increased colorectal cancer cell proliferation.[61]

Tumor suppressor activity was also demonstrated for some PP1 complexes. For instance, the migration and invasion-inhibitory protein (MIIP)/PP1c promoted the dephosphorylation of MIIP, which was associated with decreased metastatic ability and consequently with a good prognosis.[62] In addition, the SPN/PP1c was also identified in cervical cancer and, as in breast cancer, was responsible for decreased tumor growth.[63]

Lung cancer

The leucine-rich repeat protein SHOC-2 (SHOC2)/PP1c, AXIN/PP1c and PINCH1/PP1c were identified in lung cancer and their contribution to disease progression reported. The SHOC2 protein forms a complex with PP1c and the Ras-related protein M-Ras (MRAS), which dephosphorylates negative regulatory residues of RAF kinase, maximizing tumor growth and drug resistance activities of RAF-ERK pathway.[64,65] The Wnt-β-catenin pathway has also a key role in cancer development and AXIN1/PP1c-mediated dephosphorylation of AXIN1 contributes to the activation of this signaling pathway and consequent lung tumor growth.[66,67] Moreover, as described for cervical and colorectal cancer, PINCH1/PP1c was implicated in radiation therapy resistance.[49]

Some PP1 complexes also seems to predominantly inhibit lung cancer progression. The SNP/PP1c complex, also described for breast and colorectal cancer, was associated with better prognosis.[68] The protein 4.1N also binds PP1c and positively regulates its activity. This complex was reported to inactivate JNK pathway, a signaling cascade with important roles in cancer pathogenesis, thus inhibiting lung cancer progression.[69,70]

Prostate cancer

Various PP1 complexes have been described in prostate cancer and most of them were associated with tumor promoter activities. Both B-RAF and the androgen receptor (AR) are substrates of PP1α, and their activities are positively regulated by PP1c-mediated dephosphorylation. While B-RAF/PP1c activates MAPK, promoting PCa cancer invasiveness, the AR/PP1c stabilizes AR, increasing its transcriptional activity, contributing to PCa progression.[71–73] Increased AR transcriptional activity is also mediated by the PP1R14C/PP1c complex, which downregulates the MLCP holoenzyme, a negative regulator of AR activity.[74] Moreover, FER, CAV1 and NIPP1 bind to and inhibit PP1c and these complexes are common to other types of cancer. Herein, FER/PP1c complex was demonstrated to promote cell cycle progression possibly by inhibition of PP1c-induced dephosphorylation of RB.[31] The CAV1/PP1c complex was associated with decreased PCa cells apoptosis.[75] Finally, NIPP1/PP1c, whose role in cervical cancer is controversial, was found to contributed PCa cells migration.[57]

In contrast to the above mentioned studies, in PCa, MIIP/PP1c complex was found to promote MIIP dephosphorylation and facilitate the dephosphorylation of AKT. This mechanism results in suppression of the oncogenic AKT-mTOR pathway and consequent inhibition of PCa cell growth.[76]

Other types of cancer

Some PP1 complexes, previously described herein, were identified in other types of cancer. Indeed, NIPP1/PP1c and YAP2/PP1c were reported in ovarian cancer and TAZ/PP1c in thyroid cancer.[27,77,78] The role of these complexes in this malignancy are in the same line as previously referred. Nevertheless, in ovarian cancer the interactions of NIPP1 with PP1c were suggested to be promoted by OCT4 and were associated with increased cancer aggressiveness by inactivating the tumor suppressor RB.[77] Furthermore, the PINCH1/ PP1c complex was also identified in skin and pancreatic cancer and a similar tumor promoter function was described.[49] The SHOC2/ PP1c and SDS22/PP1c were also characterized in general models of cancer.[79,80]

The protein phosphatase 1 regulatory subunit 12A (MYPT1) and dual specificity mitogen-activated protein kinase 1 and 2 (MEK 1,2) form a complex with PP1c and are PP1c substrates.[81–83] The effect of MYPT1/ PP1c as tumor suppressor or promoter is controversial. While in liver cancer this complex negatively regulated the oncogene PRTM5, leading to

tumor growth suppression,[81] in ovarian cancer MYPT1/ PP1c promoted metastasis through YAP1 dephosphorylation, which is mediated by platelets.[82] Moreover, mortalin promotes the MEK1,2/PP1c interaction in skin and pancreatic cancers, promoting cancer cells proliferation.[83]

The α7 nicotinic acetylcholine receptor (α7nAChR) recruits PP1c and facilitates the progression of liver cancer, which is mediated by the TRAF6/ NF-kB cascade.[84] The phosphatase and actin regulator 1 (PHACTR1) is also a regulatory subunit of PP1c and the PHACTR1/PP1c complex promoted melanoma cells invasiveness by controlling the actomyosin assembly.[85] Furthermore, protein phosphatase 1 regulatory subunit-14A (CPI-17) and -1A (PP1R1A) inhibited PP1c activity, contributing to pancreatic cancer cells proliferation and Ewing sarcoma pathogenesis, respectively.[86,87] A role in specifically inhibiting the dephosphorylation of histone H3 was proposed to mediate CPI-17/PP1c tumor promoter activity.[86]

Modulation of PP1 complexes in cancer treatment

Targeting PP1 complexes

The targeted interference of protein phosphorylation mechanisms has long been considered a potential approach in the treatment of several diseases, including cancer. Of the various enzymes intimately involved in such events, protein kinases emerged as the first generic target for anticancer therapies.[88] Despite the treatment resistance associated with various kinase inhibitors, and the widely variable therapeutic response observed across patients, several kinase inhibitors have been approved by Food and Drug Administration (FDA) for the treatment of malignancies, whilst many more are in clinical trials.[5] The clinical success of drugs targeting protein kinases further propelled efforts to manipulate the activity of protein phosphatases, enzymes which counteract the kinase-induced phosphorylation of intracellular proteins. Despite the initial difficulties in studying and targeting phosphatases, drug discovery endeavours have more recently developed phosphatase-targeted compounds.[89]

The involvement of PP1c in several cancer-related cellular processes highlighted the obvious potential of targeting PP1c as an anticancer strategy. Various small molecules, including

tautomycin, have been developed to block the PP1c active site, thus inhibiting all PP1 holoenzymes.[90] PP1c inhibitors can impair cancer progression by stimulating apoptosis of prostate cancer cells.[91] Conversely, radioresistance was observed in lung cancer cells after PP1c inactivation.[92] Indeed, the clinical potential of direct interference of the active site of PP1c has been questioned. PP1c is involved in a broad range of cellular processes and there is significant active site conservation amongst different phosphatases. Thus, it is not surprising that PP1c inhibitors are associated with several unwanted toxic effects and possess limited efficacy. Nevertheless, the concept of targeting a specific PP1 complex, compromising the activity of PP1c against a specific substrate, has enabled the creation of truly selective modulators and is gaining momentum amongst the biomedical and pharmaceutical communities.[26,93]

The binding of PP1c to its interacting proteins depends on docking motifs that are always remote from the active site. The tight association of PP1c to RIPPOs is usually ensured by the combination of multiple binding sites. About 30 non-overlapping RIPPOs-binding sites were identified for PP1c which, given the higher number of existing RIPPOs (more than 200), indicated that these proteins must share PP1c docking motifs. Nevertheless, RIPPOs differ in the number and type of PP1c-binding motifs.[94] The most common is the RVxF motif (consensus sequence: [HKR]-[ACHKMNQRSTV]-V-[CHKNQRST]-[FW]), which is present in about 70% of RIPPOs and is crucial for their binding to PP1c.[95,96] Several additional common PP1c binding motifs were also identified, namely SILK and MyPhoNE motifs. The SILK motif (consensus sequence: [GS]IL[RK]) always appears N-terminal to the RVxF sequence, at the opposite side of the PP1c active site and was previously identified in at least seven PP1c interactors.[95] First identified in Myosin phosphatase-targeting subunit 1 (MYPT1), MyPhoNE motif (consensus sequence: RXXQ[VL][KR]X[YW]) was further detected in at least six other RIPPOs.[97] This motif is present in the N-terminal of RIPPOs, suggesting an involvement in RIPPOs isoform selection since isoforms mainly differ in N- and C-terminal. However, the interaction of PP1c with its interacting proteins in an isoform-dependent manner is rather unknown.[95,98]

Targeting PP1c-binding motifs, with the goal of disrupting or stabilizing PP1c/RIPPOs interactions has become a reality, with promising results. Structural insights into PP1 complexes suggested the potential of small molecule compounds to compete with specific

PP1c docking motifs for binding to PP1c.[25,99] Thus, they can be used to selectively disrupt one or more PP1 complexes, with possible therapeutic effects. Even compounds that interfere with the RVxF motif were associated with high selectivity, which was unpredictable considering the relative abundance of this docking motif. This may be explained by the holoenzyme-dependent importance of each motif.[14,25,99] Small molecule compounds were considered versatile since they can either disrupt or stabilize PP1 complexes. Protein-protein interactions stabilizers function as "molecular glue", increasing the affinity and stability of the complex. Despite the promising results obtained with both natural and synthetic stabilizers, they are under-represented in drug discovery.[100–102] The main challenge of stabilizing a protein complex is that it requires the simultaneous targeting of at least two proteins.[103] More recently, larger molecules, including peptides, emerged in an attempt to overcome the limitations associated with small molecule compounds. Some of these peptides have been chemically optimized to produce stable and cell penetrating homologues. Indeed, peptides that mimic the PP1c-binding motifs of different RIPPOs have been developed to disrupt PP1 complexes with promising results.[104–106] The main advantages of these compounds, compared with more conventional small molecules, are their reduced immunogenicity, improved safety, and high selectivity and potency.[107]

Modulation of PP1 complexes in cancer: the state of the art

In recent years, the targeted modulation of PP1 complexes has gained increased attention among the scientific community as a potential approach in cancer treatment. Indeed, several studies using different strategies proved successful in targeting PP1 holoenzymes, consequently delaying cancer progression. Some of these strategies have been subsequently evaluated in animal models with promising results. The main studies in this topic are described in the following sections and summarized in **Table 2** and **Figure 2**.

HDACs/ PP1c complexes

Histone deacetylases (HDACs) are enzymes responsible for removing acetyl groups from lysine residues in histone and nonhistone proteins and are generally expressed in almost all human tissues.[108] Eighteen HDAC isoforms (HDAC1-11 and SIRT1-7) grouped in four classes (I-IV), which differ in sequence homology, compose this protein family.[109] A role in modulating a range of key cellular processes has been attributed to HDACs. Indeed, evidence of the

involvement of these proteins in the regulation of metabolism[110] and senescence,[111] in transcriptional repression,[112] in cell cycle control[113], in the induction of chaperone function[114] and angiogenesis,[115] in either promotion or restriction of apoptosis[116] and autophagy[117,118] (depending on the type), among others, have been reported.

The perturbation of acetylation homeostasis is common to almost all types of cancer.[119] Dysregulation of HDACs has been considered the main contributor to these alterations in acetylation status.[120] Indeed, dysregulated expression and/or activity of HDACs is common to different types of cancer.[121] In general, HDACs are considered cancer promoters and upregulation of these proteins is associated with advanced disease stages and poor outcomes.[122–125] Nonetheless, some class III HDACs has been related to tumor suppressor activity, namely SIRT2 and SIRT6.[126] By deacetylating histones, dysregulated levels of HDACs alter the transcription of oncogenes and tumor suppressor genes, and modulate chromatin remodelling and nuclear architecture, thus contributing to cancer development and progression.[127] Nonhistone cellular substrates are also affected by HDACs dysregulation, which can also contribute to tumorigenesis, tumor progression and metastasis.[128]

To perform their roles, HDACs interact with different proteins, including PP1c. In fact, the interaction between HDAC1/2 and PP1c was confirmed both *in vitro*[129] and *in vivo.*[130] Dephosphorylation of HDAC1/2 by PP1c was demonstrated, but the net effect of PP1c-dephosphorylation upon the activity of HDACs remains unclear.[129] Inhibition of PP1c by okadaic acid and consequent hyperphosphorylation of HDAC1 and 2 was associated with increased activity of HDACs, mainly through disruption of complexes between HDACs and their co-repressors.[129] These findings support the general concept that phosphorylation of HDAC1 and 2 is correlated with increased enzymatic activity.[131] Conversely, other authors have suggested that the enzymatic activity of HDAC1 is unrelated to its association with PP1c.[132] The effect of the HDACs/PP1c complex upon the activity of PP1c is also controversial and seems to depend on the substrate. For instance, the HDAC1/PP1c complex was associated with inhibition of PP1c-mediated AKT dephosphorylation (**Figure 2A**).[133] These alterations in protein phosphorylation and acetylation status caused by the interaction between HDACs and PP1c seems to contribute to the ability of HDACs to promote cell growth and malignant transformation.[134]

Given the aforementioned HDACs' functions, they have been considered promising drug targets in cancer.[135] Several HDAC inhibitors (HDACi) have been developed over the last years for several types of cancer. Some of these, including Vorinostat (SAHA),[136] Panobinostat (LBH589)[137] and Romidepsin,[138] have already been approved by the FDA and EMA. Others, exemplified by Quisinostat,[139] CUDC-101[140] and Valproic acid[141] are currently in clinical trials. The disruption of HDACs/PP1c complexes seems to be one of the major mechanisms contributing to the beneficial effects of some HDACi in cancer therapies (**Figure 2A**). Trichostatin A (TSA), the first HDACi proven to disrupt PP1 complexes, positively delayed cancer progression. TSA is a broad-spectrum HDACi, able to disrupt interactions of several HDACs with PP1c. The blockage of HDAC1,6/PP1c interactions in glioblastoma and prostate cancer cells resulted in proliferation's suppression,[133] while disruption of HDAC1,2/PP1c complexes induced apoptosis in breast cancer cells.[142,143] When used in combination with C6-ceramide, TSA synergistically inhibited the growth of ovarian and pancreatic tumors, both *in vitro* and *in vivo.*[144] The hydroxamic acid LBH589 reversed the rapamycin-resistance of lymphoma cells by supressing HDAC/PP1c interaction[145] and also induced ERK-dependent arrest of prostate cancer cells by blocking HDAC6/PP1c interaction.[146] Moreover, the disruption of the HDAC6/PP1c complex was also achieved by treating melanoma cells with HDAC-inhibitor (S)-8, resulting in growth arrest and apoptosis.[147] Intraperitoneal injection of (S)-8 in mice xenografts demonstrated a good safety profile.[147] Finally, HDAC/PP1c disruption by SAHA led to increased cisplatin-induced apoptosis of oral cancer cells.[148] Thus, the beneficial effects of different HDACi, either as single agents or in combination with other chemotherapeutic drugs, was apparent in several types of cancer.

AKT/ PP1c complex

The AKT kinase family comprises three highly homologous isoforms (AKT1-3) of serine-threonine kinases.[149] Increased levels of AKT were observed in different types of cancer, including breast, prostate and ovary cancers, and were associated with a role in oncogenic transformation and a correspondingly poor prognosis.[150] Indeed, AKT is considered a node of several signaling pathways, most of them with important roles in cancer development and progression.

Most of the roles of AKT are mediated by phosphorylation of a wide range of downstream effectors. One of these effectors is the Bcl2-associated agonist of cell death (BAD), which is

phosphorylated by AKT, inactivating its ability to induce apoptosis, thus promoting cell survival.[151] Cell survival is also promoted by AKT-induced phosphorylation and consequent inhibition of Forkhead transcription factors, including Forkhead box protein O3 (FKHRL1).[152] CREB is also a downstream effector of AKT, which, through induction of CREB phosphorylation, enhances the transcription of genes critical for cell survival.[153] Several metabolic enzymes, mainly involved in glucose metabolism, are modulated by AKT phosphorylation. Glycogen synthase kinase-3 (GSK-3) is one example which is inactivated when phosphorylated.[154,155] The influence of AKT upon tumor angiogenesis is also well characterized and is associated with phosphorylation and consequent activation of endothelial nitric oxide.[156] AKT phosphorylation also activates various oncogenic factors, including Inhibitor of nuclear factor kappa-B kinase subunit alpha (IKKα),[157] and is an effector of one of the major oncogenic signaling pathways: PI3K/AKT/mTOR. This signaling pathway is responsible for promoting cell proliferation and survival and preventing apoptosis.[158]

Due to the previously mentioned roles, AKT has been considered an oncogene and a potential drug target for cancer therapy.[159] Thus, efforts to identify specific AKT inhibitors have intensified. Some of these have proven successful by inhibiting AKT and delaying cancer progression. For example, Verrucarin J significantly inhibited AKT in metastatic colon cancer cells, reducing tumor growth and initiating apoptotic signaling.[160] MK-2206 also successfully inhibited AKT, resulting in decreased glioblastoma cells migration.[161] The main limitation associated with this type of inhibitors is their low selectivity since most of their binding pockets are conserved among different kinases. For instance the ATP-binding pocket of AKT is highly conserved among kinases within the human cell[162].

It is well established that phosphorylation of AKT at specific serine and threonine residues is essential for its activity.[163] Thus, dephosphorylation has been considered a potential strategy to inactivate AKT. Promoting the interaction of AKT with PP1c, a major phosphatase that dephosphorylates in threonine 450 and inactivates AKT,[164] has shown promising results in different types of cancer (**Figure 2B**). Targeting this interaction seems to be a more selective approach to interfere with AKT activity. Sphingosine (SPH) was the first compound directly associated with the promotion of AKT/ PP1c interaction. Indeed, when leukemia cells were incubated with SPH, cells apoptosis was evident. The prime signaling event responsible for this effect was PP1c-dependent dephosphorylation of AKT.[165] Moreover, inhibition of

proliferation and apoptosis induction was also noted in melanoma cells incubated with C6-ceramide. PP1c-dephosphorylation of AKT was identified as responsible for these effects. Non-cytotoxicity was observed after the incubation of mouse melanocytes and primary human melanocytes with C6-ceramide.[166] Osteosarcoma cell proliferation was inhibited with a combination of doxorubicin and phenoxodiol. This treatment seems to increase the cellular levels of ceramide and thus promote the interaction between AKT and PP1c. The combination of these compounds was further analysed *in vivo* using a mice xenograft model and, in accord with *in vitro* results, inhibition of cell growth was observed.[167] Lastly, ZD1839 seems to indirectly promote the interaction of AKT with PP1. This compound directly inhibits ErbB2, the activity of which has been shown to inhibit PP1-dependent dephosphorylation of AKT. Thus, by inhibiting ErbB2, ZD1839 promotes the interaction AKT/ PP1c to achieve an anticancer effect in breast cancer cells.[168] Besides these examples, other compounds seem to promote the AKT/PP1c interaction by disrupting other PP1 complexes and therefore releasing PP1c and promoting its interaction with AKT. This is reported, for example, for some HDAC inhibitors, like LBH589, SAHA or TSA.[133,145,148]

GADD34/PP1c and EIF2α/PP1c complexes

The GADD34 protein was reported to be induced by different types of cellular stress and DNA damage to mediate cell growth arrest and induce apoptosis.[169] Indeed, in response to cellular stress, the unfolded protein response (UPR) is activated, triggering the rapid translation of GADD34, essential for UPR progression. A structural homologue of GADD34 – CreP, although is unchanged by stress, plays also a key role in the regulation of UPR.[170] Due to the accumulation of misfolded proteins in the endoplasmic reticulum (ER), UPR results in ER stress.[171] In an attempt to maintain homeostasis, cells respond to stress by activating several adaptive mechanisms. One of the most common is the PRKR-like ER kinase (PERK)-mediated phosphorylation of the transcription factor eIF2α, a mechanism which inhibits the translation of general proteins to promote cellular recovery.

The action of GADD34 is the consequence of a specific interaction with PP1c mediated by two binding motifs, namely the PP1c-binding motif KVRF and a RARA sequence.[134] The CReP shares sequence homology in the C-terminal PP1-binding domain with GADD34.[170] Thus, GADD34 and CreP have been considered regulatory subunits of PP1c which, by binding to PP1c, can modulate both the activity and substrate specificity of the phosphatase.[172] The best

described substrate of these complexes is eIF2α, which, by an action opposite to that of PERK, is dephosphorylated in serine 51 by the GADD34/PP1c and CReP/PP1c complexes.[172,173] Dephosphorylation restores the function of eIF2α to promote general protein synthesis and inhibit cellular recovery; this activity which may subsequently induce cellular apoptosis (**Figure 2C**).[174] In addition to eIF2α, other complexes substrates have been identified, but their role(s) are currently not well defined.[174]

UPR activation and ER stress have been documented in many cancers. For example, various UPR components were associated with lung cancer tumorigenesis.[175] A more severe prognosis was observed in patients with increased levels of UPR-associated proteins, including GADD34.[176] More specifically, GADD34 has been reported to promote lung tumor growth,[177] inhibit TNF-related apoptosis-inducing ligand (TRAIL)-induced liver cancer cell apoptosis[178] and upregulate pro-inflammatory mediators production leading to increased tumor burden.[179] UPR seems to be an important mechanism to maintain cancer cells malignancy.[180] While the blockage of translation caused by the GADD34/PP1c complex may provide either survival or death signals, depending on the context, cancer cells seem to have adapted to gain advantage from UPR, avoiding apoptosis.[181] Therefore, cancer cells undergo an active stress response and the GADD34/PP1c complex seems to contribute to tumor cell malignancy. In contrast to normal cell, this complex does not induce apoptosis in transformed cells. Inhibition of GADD34 and CReP were also associated with increased levels of phosphorylated eIF2α and consequent breast cancer cells apoptosis.[182]

The important role(s) of UPR and ER stress in cancer has promoted the development of various UPR-targeted cancer therapeutics. Indeed, several UPR-targeted drugs have been approved or are in clinical trials for the treatment of different types of cancer.[180] The GADD34/PP1c interaction is also considered a cancer therapeutic target and its disruption has been validated using different strategies (**Figure 2C**).[51,183] Both the small mitoxantrone and a GADD34-derived peptide were able to disrupt the interaction between GADD34 and PP1c in cervical cancer cells, with a consequent increase of phospho-eIF2α. In both cases, the reduction of this complex triggered calreticulin exposure, a common feature of adaptive anticancer immune responses. Nevertheless, in contrast to mitoxantrone, the GADD34-peptide failed to stimulate apoptosis.[51] Another peptide inhibitor of the GADD34/PP1c complex, that targets the non-catalytic binding site of GADD34 to PP1, was coupled to a

homing peptide to direct the conjugate to cancer cells.[178] This peptide inhibitor is likely to also disrupts the CReP/PP1c complex due to the shared homology in the PP1 binding site between GADD34 and CReP. When tested in combination with chemotherapeutic drugs (5-fuorouracil and docetaxel), in colon and breast cancers and fibrosarcoma, this peptide successfully disrupted the interaction and enhanced the anticancer activity of chemotherapeutic drugs *in vitro* and *in vivo*, inhibiting the tumor growth and increasing mice survival.[183] With the objective to impair UPR in cancer cells, the inhibition of eIF2α/PP1c was also evaluated. A small molecule (OSU-03012) was able to disrupt this interaction, thereby increasing eIF2α phosphorylation. When in combination with lapatinib, an anticancer drug, OSU-03012 sensitized breast cancer cells to lapatinib-induced cell death.[184]

Other complexes

The mechanism of some FDA approved chemotherapeutic drugs seems to involve the dissociation of PP1 complexes. One example is the taxane Paclitaxel, which releases PP1c from the mitogen-activated protein kinase (JNK)/Bcl-2/PP1c complex.[185] Bcl-2 is an antiapoptotic protein, which may be inhibited by multi-site phosphorylation. Phosphorylation status, and consequently the activity, of Bcl-2 is controlled by a different mechanism involving both JNK and PP1c, with which it forms a tripartite complex in mitochondria.[186] By inhibiting the interaction of JNK/Bcl-2 with PP1c, paclitaxel increases the phosphorylation of Bcl-2, inhibiting its antiapoptotic activity and thus inducing breast and cervical cancer cell apoptosis.[185] Furthermore, Cytarabine (Ara-C) is able to disrupt the serine/threonine-protein phosphatase 1 regulatory subunit 10 (PNUTS/PP1c) complex.[187] PNUTS has been considered an oncogene, mainly by sequestering Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase (PTEN), a tumor suppressor gene.[188] Increased levels of PNUTS were evident in many cancers and it has been associated with poor prognosis.[188,189] PNUTS forms a complex with PP1c and this interaction decreases the PP1c-induced dephosphorylation of several exogenous substrates, including RB, thus inactivating RB tumor suppressor activity.[190] Ara-C, by disrupting PNUTS/PP1c interaction led to dephosphorylation of RB and consequently to breast cancer cells apoptosis.[187]

Cell division cycle associated 2 (CDCA2) was found to be overexpressed in many cancers, contributing to cancer progression.[191,192] Through interaction with PP1c, CDCA2 suppresses the DNA damage response activation, thus contributing to malignancy.[193] A peptide based on

the non-catalytic RVTF-binding motif was used to disrupt the RVTF-mediated interaction between PP1c and CDCA2 in Xenopus egg extracts, a well-recognized *in vitro* and *in vivo* model in cancer research.[194] The blockage of CDCA2/PP1c led to ataxia telangiectasia mutated (ATM)-mediated phosphorylation of Chk1 and Smc1, resulting in decreased levels of DNA damage.[193]

Lastly, the expression of cofilin (CFL) is also increased in many cancers, which is again associated with relatively poor prognosis. Indeed, its involvement in cell migration and tumor invasion was demonstrated.[195] The phosphorylation status of CFL is a key determinant of its activity. Increased phosphorylation of CFL seems to be related to cancer occurrence and invasiveness.[196] Accordingly, CFL dephosphorylation was associated with the improvement of cancer outcomes. Indeed, allyl isothiocyanate (ATIC), a plant-derived natural small molecule, induced leukemia cell apoptosis, mainly through promoting CFL interaction with PP1c. This interaction resulted in the dephosphorylation of CFL and its consequent translocation to mitochondria to induce intrinsic apoptosis.[197]

Conclusions and future challenges

Even though it is not yet a well-studied and established topic, the modulation of PP1 complexes has recently emerged as a promising strategy for cancer treatment. Limited knowledge of PP1 complexes' specific roles in tumorigenesis, and the molecular characterization of only a small proportion of all PP1 complexes, have necessarily limited this approach. Indeed, to the best of our knowledge, only 38 different PP1 complexes have been functionally characterized in cancer models, and even some of their roles are not fully understood. Of the common human malignancies, the largest number of PP1 complexes have been characterized in breast cancer. Such efforts are further compromised by the contradictory roles demonstrated for different PP1 complexes; some seemingly function as tumor suppressors whilst others promote tumor development. These findings highlight the importance of understanding the how the balance between various PP1 complexes contribute to cancer progression. Moreover, some complexes, including PINKCH1/PP1c, SPN/PP1c and NIPP1/PP1c, seem to be common to different types of cancer, whilst the actions of others may be specific to a particular tumor type. Though many studies do not confirm a PP1c isoform, a predominance of PP1α is evident in the literature, suggesting this

isoform might be most significant target to control tumor development and progression. The same data suggest an isoform-dependent interaction of PP1c with RIPPOs.

Several small molecules were found to successfully disrupted HDACs/PP1c holoenzymes, thus supporting their anticancer activity. A potential mechanism to overcome the resistance to some chemotherapeutic drugs was also proposed for some of them. Conversely, various small molecules have also been developed to stabilize the AKT/PP1c complex, a rather more challenging approach. Thus, several compounds either directly or indirectly inactivated AKT by promoting its interaction with PP1c with beneficial results for application in cancer treatment. The GADD34/PP1c interaction was interrupted by a small molecule and by different peptides that mimic the PP1c binding motifs, which demonstrated potential either as a monotherapy or as an adjunct of chemotherapeutic drugs. The potential of peptides has increased in last years with more and more successful compounds being tested. Despite the potential of most of the tested compounds, pre-clinical and clinical validation are still required for most of them to confirm their beneficial effects. Moreover, we suggest that the combination of some of these compounds, thus interfering with more than one PP1 complex, may lead to improved outcomes in cancer treatment.

In conclusion, the modulation of PP1 complexes is a promising approach for cancer treatment. The precise identification of PP1 complexes in several cancer models, and their molecular characterization both *in vitro* and *in vivo*, is imperative to elucidate their therapeutic potential. We anticipate that structure-based studies of PP1 complex interfaces will also contribute to the development of more effective strategies to modulate these challenging targets.

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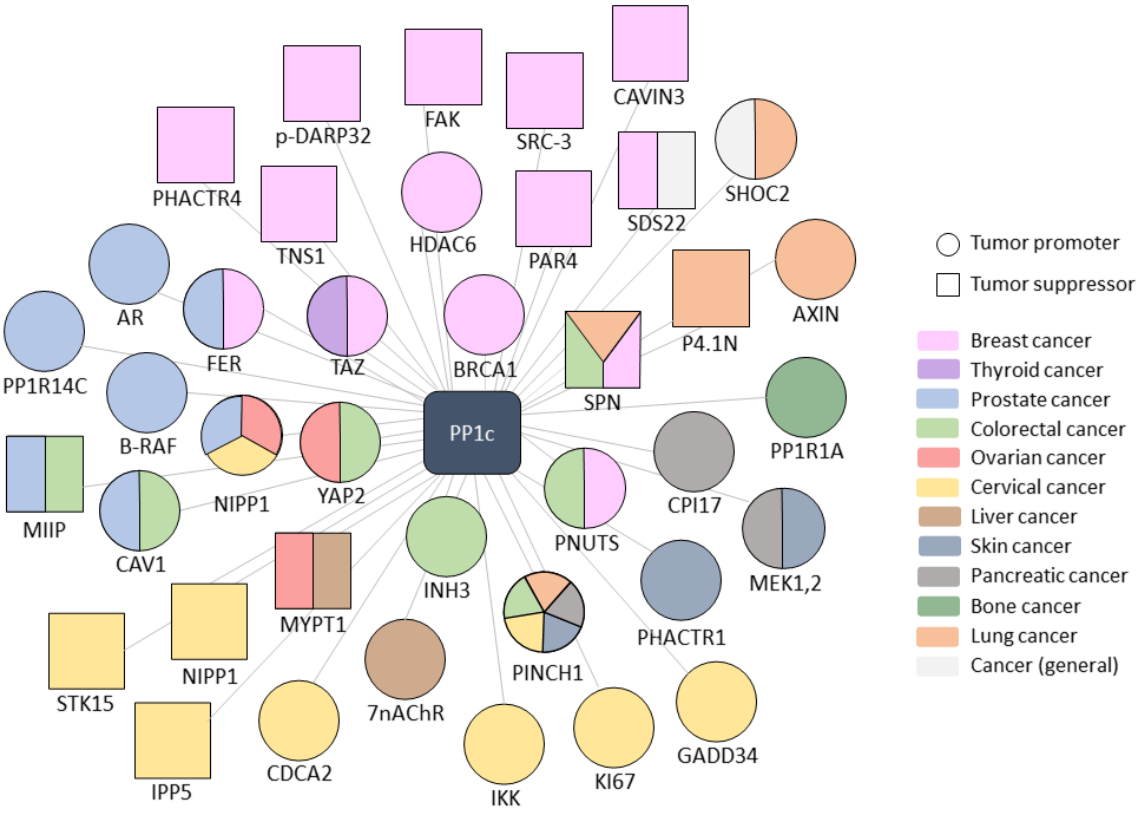

Figure 1: PP1 complexes characterized in different types of cancer. The lines represent the interactions of PP1c with its interactors. The interactors that were associated with tumor development are represented as circles, while the tumor suppressor interactors are represented as squares. The different colours represent the different types of cancer.

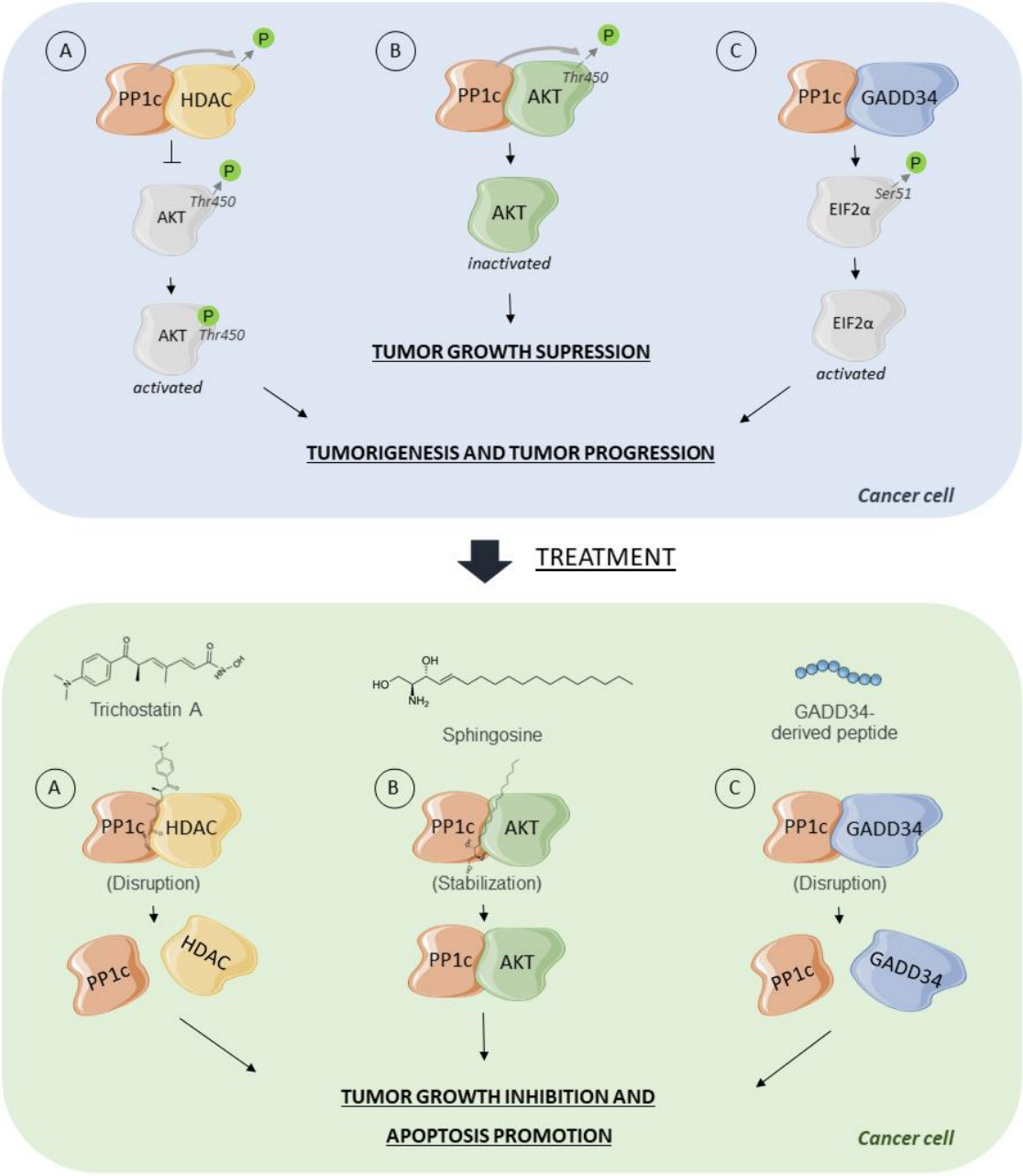

Figure 2: **Summary of the mechanisms through which HDACs/PP1c and GADD34/PP1c induce tumorigenesis and tumor progression, and AKT/PP1c supresses tumor growth (top). At the bottom, the output of the disruption or stabilization of these PP1 complexes by different strategies**. A: The HDAC/PP1c complex leads to HDAC dephosphorylation and inhibits the dephosphorylation of AKT, activating it, which promotes tumorigenesis and tumor progression (top); The disruption of this interactions by Trichostatin A inhibits tumor growth. A similar mode of action is observed with C6-ceramide+TSA, LBH589, HDAC inhibitor (s)-8 and SAHA (bottom); B: The AKT/PP1c complex results in AKT dephosphorylation and consequent

inhibition, suppressing tumor growth (top); The stabilization of this complex by sphingosine (SPH) inhibits tumor progression. Phenoxodiol and C6-ceramide have a similar effect (bottom); C: The GADD34/PP1c complex dephosphorylates EIF2α, activating it to promote tumor development and progression (top); The blockage of this complex by a GADD34-derived peptide inhibits tumor progression. A similar mode of action is observed with the small molecule anthracyclin mitoxantrone (bottom).

Table 1: Summary of the PP1 complexes characterized in different types of cancer. The PP1c isoform involved, the effect of RIPPO on PP1c activity and the role of the complex in cancer are also included.

Type of cancer	PP1 complex	PP1c isoform	Cell line	Tumor promoter/ suppressor	Effect of RIPPO on PP1c activity	Role in cancer	Reference
Breast cancer	TAZ/ PP1c	PP1α	MCF10A	Tumor promoter	TAZ is a substrate of PP1c	promote breast cancer cells proliferation	[27]
	BRCA1/ PP1c	PP1β	HEK293T		BRCA1 is a substrate of PP1 and targets PP1c to dephosphorylate specific substrates	role in the development of breast cancer	[12]
	PNUTS/ PP1c	PP1α, β, ϒ	MDA-MB-231 and HS 578T		PNUTS activated PP1c activity against a specific substrate	induce breast cancer cells proliferation	[29]
		-	MCF7		PNUTS inhibits PP1c activity towards a specific substrate	decrease breast cancer cells apoptosis	[30]
	FER/ PP1c	PP1α	MDA-MB-231		FER inhibits PP1c activity against a specific substrate	promotes the breast cancer cell cycle progression	[31]
	HDAC6/ PP1c	-	R2d		HDAC6 inhibits the activity of PP1c against a specific substrate	involved in phthalate-induced tumorigenesis and metastasis	[32]
	PHACTR4/ PP1c	PP1α, β, ϒ	HMECs	Tumor suppressor	PHACTR4 activates PP1c against a specific substrate	decrease breast cancer cells proliferation	[34]
	SDS22/ PP1c	-	MCF7, MDA-MB-231, T47D		SDS22 facilitates PP1c activity against a specific substrate	increase breast cancer cells apoptosis	[35]
	SPN/ PP1c	PP1α	T47D and MDA-MB-468		SPN inhibits PP1c activity against a subset of substrates	decrease tumorigenic proprieties of breast cancer cells	[36]
	p-DARP-32/ PP1c	-	HB2 and MCF-7		p-DARP-32 inhibits PP1c activity against a specific substrate	inhibiting breast cancer cells migration	[37]
	TNS1/ PP1c	PP1α	MDA-MB-231		TNS1 is a substrate and regulatory subunit of PP1c	supressed migration and invasion (essential for metastasis)	[39]
	FAK/ PP1c	-	MDAMB468 and MDAMB231		FAK is a substrate of PP1c	decrease migration and invasion of breast cancer cells	[41]
	SRC-3/ PP1c	-	MDA-MB-231		SRC-3 is a substrate of PP1c	inhibits SRC-3-enhanced cell proliferation and metastasis	[42]
	CAVIN3/PP1c	PP1α	MCF-7		CAVIN3 inhibits PP1c activity	stimulated apoptosis in response to UV treatment	[45]

	PAR4/ PP1c	PP1β	Primary and recurrent BCa cell lines		PAR4 is a regulatory subunit of PP1c	resensitizes tumor to chemotherapy	[46]
Cervical cancer	Ki-67/ PP1c; Repo-Man/ PP1c	PP1Υ	HeLa	Tumor promoter	Ki-67 and Repo-Man are re regulatory subunits of PP1c	target of both holoenzymes proposed as cancer therapeutic target	[47]
	PINCH1/ PP1c	PP1α	HeLa		PINCH1 inhibits PP1c activity against a specific substrate	enhance tumor cells resistance to radiation therapy	[49]
	IKKα, β, Υ/ PP1c	-	HeLa		-	contributes to cancer cells survival	[50]
	GADD34/ PP1c	-	HeLa		GADD34 facilitates PP1c activity against a specific substrate	Inhibits cancer cells apoptosis	[51]
	STK15/ PP1c	-	HeLa	Tumor suppressor	STK15 inhibits PP1c activity	anomalous chromosome segregation during mitosis	[53]
	IPP5/ PP1c	-	HeLa		IPP5 inhibits PP1c activity	supress tumor growth and progression of cervical carcinoma	[55]
	NIPP1/ PP1c	-	HeLa	Tumor suppressor	NIPP1 inhibits PP1 and titrated away PP1c from other mitotic interactors.	inhibits colony formation and tumor growth	[56]
	NIPP1/ PP1c	-	HeLa	Tumor promoter	NIPP1 inhibits PP1c	contribute to migratory proprieties of cervical cancer cells	[57]
Colorectal cancer	INH3/ PP1c	-	SW480 and SW620	Tumor promoter	INH3 inhibits PP1c activity	mediates hypoxia-induced metastasis formation	[58]
	CAV-1/ PP1c	-	HCT116		CAV-1 inhibits PP1c activity	increase p-Akt and KLK6 secretion; involved in migration and invasion and associated to poor prognosis	[60]
	PNUTS/ PP1c	-	MCF7 and HCT116		PNUTS inhibits PP1c activity toward a specific substrate	decrease breast and colon cancer cells apoptosis	[30]
	PINCH1/ PP1c	PP1α	DLD1, HCT15, and HCT116		PINCH1 inhibits PP1c activity against a specific substrate	enhance tumor cells resistance to radiation therapy	[49]
	YAP2/ PP1c	-	HT-29 and SW620		YAP2 is a substrate of PP1c	induced colon cancer cells proliferation	[61]
	MIIP/ PP1c	-	SW480 and SW620	Tumor suppressor	MIIP is a substrate of PP1c	decrease metastatic ability of tumor cells	[62]
	SPN/ PP1c	PP1α	COLO205, HT29 and SW480		SPN is a regulatory subunit of PP1c	decrease tumor growth	[63]

Lung cancer	SHOC2/ PP1c	-	RAS-mutant NSCLC cells	Tumor promoter	SHOC2 targets PP1c to specific substrates	tumorigenic proprieties; proposed as an attractive therapeutic target for cancer	[64]
	AXIN/ PP1c	-	H1299 and SK-MES-1		AXIN is a substrate of PP1c	activate Wnt/ β-catenin signaling, promoting tumor growth	[67]
	PINCH1/ PP1c	PP1α	A549 and H1299		PINCH1 inhibits PP1c activity against a specific substrate	enhance tumor cells resistance to radiation therapy	[49]
	SPN/ PP1c	PP1α, β, ϒ	(Calu-1, HTB59, H520 and H226	Tumor suppressor	SPN targets PP1c to a specific substrate	associated with a better prognosis	[68]
	Protein 4.1N/ PP1c	-	H1299, H460, SK-MES-1 and 95C		Protein 4.1N positively regulated PP1c activity	inactivate JNK-c-Jun signaling pathway and decrease expression of downstream metastatic targets	[69]
Prostate cancer	B-RAF/ PP1c	PP1α	PC-3	Tumor promoter	B-RAF is a substrate of PP1c	promoted prostate cancer cells invasiveness	[71]
	AR/ PP1c	PP1α	LNCaP and CWR-RV1		AR is a substrate of PP1c	increase AR-mediated gene transcription	[72]
			LNCaP and C4-2		AR is a substrate of PP1c	increase AR transcriptional activity	[73]
	PP1R14C/ PP1c	PP1β	LNCaP		PP1R14C is a regulatory subunit of PP1c	increase prostate cancer cells proliferation	[74]
	FER/ PP1c	PP1α	PC3		FER inhibits PP1c activity	promotes the prostate cancer cell cycle progression	[31]
	CAV-1/ PP1c	-	LNCaP		CAV-1 inhibits PP1c activity	decrease prostate cancer cells apoptosis	[75]
	NIPP1/ PP1c	-	PC-3		NIPP1 inhibits PP1c	contribute to migratory proprieties of prostate and cancer cells	[57]
	MIIP/ PP1c	PP1α	LNCaP, C4–2, 22Rv1 and PC3	Tumor suppressor	MIIP is a substrate of PP1c and facilitates the dephosphorylation of a specific substrate by PP1c	inhibits the growth of prostate cancer	[76]
Liver cancer	MYPT1/ PP1c	-	HepG2	Tumor suppressor	MYPT1 targets PP1c to its substrates	supress tumor growth	[81]
	7nAChR/ PP1c	PP1ϒ	Huh7, SMMC-7721, HepG2, QGY-7703, and HEK-293T	Tumor promoter	7nAChR mediates the recruitment of PP1c	promoted hepatocellular carcinoma cells proliferation	[84]
	NIPP1/ PP1c	-	NIH-OVCAR-3		NIPP1 inhibits PP1c	promoting tumor growth	[77]

Ovarian cancer	YAP2/ PP1c	PP1α	Ovarian cancer cells (ATCC)	Tumor promoter	YAP2 is a substrate of PP1c	increase YAP2 pro-survival activity of cancer cells	[78]
	MYPT1/ PP1c	-	OVCA432 and MDAH-2774		MYPT1 targets PP1c to a specific substrate	promoted metastasis through inducing resistance to apoptosis	[82]
Skin cancer	PINCH1/ PP1c	PP1α	A431	Tumor promoter	PINCH1 inhibits PP1c activity against a specific substrate	enhance tumor cells resistance to radiation therapy	[49]
	MEK1,2/ PP1c	PP1α	SK-MEL 28, SK-MEL 1 and RPMI-7951		MEK1,2 is a substrate of PP1c	promoted melanoma cells proliferation	[83]
	PHACTR1/ PP1c	-	CHL-1		PHACTR1 is a regulatory subunit of PP1c	promoted melanoma cells invasiveness	[85]
Pancreatic cancer	PINCH1/ PP1c	PP1α	d PaTu and MiaPaCa2	Tumor promoter	PINCH1 inhibits PP1c activity against a specific substrate	enhance tumor cells resistance to radiation therapy	[49]
	MEK1,2/ PP1c	PP1α	PANC-1		MEK1,2 is a substrate of PP1c	promoted pancreatic cells proliferation	[83]
	CPI-17/ PP1c	-	(L3.6pl, AsPC-1 and BxPC-3		CPI-17 inhibits PP1c activity	promote proliferation of pancreatic cancer cells	[86]
Thyroid cancer	TAZ/ PP1c	PP1α	HEK293T and HeLa	Tumor promoter	TAZ is a substrate of PP1c	promote thyroid cancer cells proliferation	[27]
Bone cancer	PP1R1A/ PP1c	-	TC71 and EWS502	Tumor promoter	PP1R1A inhibits PP1c activity	required for tumor formation and Ewing sarcoma pathogenesis	[87]
Cancer (general)	SHOC2/ PP1c	-	293T	Tumor promoter	SHOC2 targets PP1c to specific substrates	promotes tumor development through activation of MAPK pathway	[79]
	SDS22/ PP1c	-	drosophila	Tumor suppressor	SDS22 is a regulatory subunit of PP1c	supress tumorigenic growth and inhibit invasive ability	[80]

PP1c: phosphoprotein phosphatase 1 catalytic subunit

Table 2: Summary of the PP1 complexes modulated (disrupted or stabilized) by either small molecules or peptides. The respective output of the complexes' modulation *in vitro* and *in vivo* (when available) are also included.

PP1 complex	Type of cancer	Type of strategy	Strategy	Modulation	Cell line	Output of modulation *in vitro*	Tested in vivo	Output of modulation *in vivo*	Refs.
HDAC1,6/ PP1c	Glioblastoma and prostate cancer	Small molecule	TSA	Disruption	U87MG and PC-3	supressed cancer cells proliferation	-	-	[133]
HDAC1,2/ PP1c	Breast cancer	Small molecule	TSA	Disruption	MCF-7	induced breast cancer cells cytotoxicity (mediated by GSK3β activation	-	-	[142]
						induced breast cancer cells apoptosis	-	-	[143]
HDAC6/ PP1c	Ovarian and pancreatic cancer	Small molecule	C6-ceramide+TSA	Disruption	CaOV3 and L3.6	decreased tumor growth	mice (xenografts)	inhibited tumor growth	[144]
HDAC/ PP1c	Lymphoma	Small molecule	LBH589	Disruption	SUDHL-6, OCI-Ly7 and OCI-Ly3	inhibited lymphoma cells survival and proliferation	-	-	[145]
HDAC6/ PP1c	Prostate cancer	Small molecule	LBH589	Disruption	LNCaP, PC-3, DU-145 and 22Rv1	induced ERK-dependent prostate cancer cells arrest	-	-	[146]
HDAC6/ PP1c	Melanoma	Small molecule	HDAC-inhibitor (s)-8	Disruption	Hs-294T and MeWo	prompted melanoma cells arrest and apoptosis	mice (xenografts)	good safety	[147]
HDAC/ PP1c	Oral cancer	Small molecule	SAHA	Disruption	HSC-3	increased cisplatin-induced apoptosis	-	-	[148]
AKT/ PP1c	Leukemia	Small molecule	SPH	Stabilization	Jurkat	induce Jurkat cells apoptosis	-	-	[165]
	Melanoma	Small molecule	C6 ceramide	Stabilization	SK-Mel2, WM-266.4, A-375 and WM-115	inhibited melanoma cells proliferation and induce caspase-dependent apoptosis	mice (xenografts)	non-cytotoxicity was observed	[166]
	Osteosarcoma	Small molecule	Phenoxodiol	Stabilization	U2OS, MG-63, and SaOs-2	combined with doxorubicin inhibited osteosarcoma cell growth	mice (xenografts)	suppressed tumor growth	[167]

	Breast cancer	Small molecule	ZD1839	Stabilization	SKBR3	demonstrated anticancer activity	-	-	[168]
GADD34/ PP1c	Cervical cancer	Small molecule	Anthracyclin mitoxantrone	Disruption	HeLa	triggered CTR exposure with consequent cells apoptosis	-	-	[51]
		Peptide	GADD34-derived peptide			triggered CTR exposure			
	Colon and breast cancer and fibrosarcoma	Peptide	GADD34-derived peptide	Disruption	several cell lines	improved the anticancer activity of chemotherapy	mice (xenografts)	reduce tumor growth	[183]
EIF2α/ PP1c	Breast cancer	Small molecule	OSU-03012	Disruption	MDA-MB-231	sensitized breast cancer cells to lapatinib-induced cell death	-	-	[184]
JNK/ BCL2/ PP1c	Cervical and Breast cancer	Small molecule	Paclitaxel	Disruption	HeLa and MCF-7	promoted cervical and breast cancer cells mitotic arrest and apoptosis	-	-	[185]
PNUTS/ PP1c	Breast cancer	Small molecule	Ara-C	Disruption	CV1 and Hs578T	promoted breast cancer cells apoptosis	-	-	[187]
CDCA2/ PP1c	Breast cancer	Peptide	RVTF peptide	Disruption	Xenopus laevis egg extract	decreased DNA damage	-	-	[193]
CFL/ PP1c	Leukemia	Small molecule	AITC	Stabilization	U937, HL-60, and Jurkat	induced leukemia cells apoptosis	mice (xenografts)	inhibited tumor growth	[198]