MHC Phosphopeptides: Promising Targets for Immunotherapy of Cancer and Other Chronic Diseases

Authors
Keira E. Mahoney, Jeffrey Shabanowitz, and Donald F. Hunt

Correspondence
js4c@virginia.edu; dfh@virginia.edu

In Brief
Although mutated antigens are currently the main focus of immunopeptidomics, disease-associated PTMs create potential targets for immunotherapeutic treatment of cancers and other diseases. We have found that many phosphorylated antigens are expressed across multiple cancers and patients. We propose that the most likely cause of this increased MHC phosphorylation is inhibition of PP2A, which is ubiquitously inhibited across cancers. If valid, a multitude of other diseases that inhibit PP2A could be targeted using the same antigen targets.

Highlights
• Inhibition of PP2A should increase presentation of phosphorylated MHC peptides.
• Dysregulation of p53 or pRb has been shown to cause inhibition of PP2A.
• Many diseases inhibit PP2A and should present similar phosphorylated MHC peptides.
• Disease and cancer-associated PTMs are promising targets for immunotherapeutics.
MHC Phosphopeptides: Promising Targets for Immunotherapy of Cancer and Other Chronic Diseases

Keira E. Mahoney¹, Jeffrey Shabanowitz¹,*, and Donald F. Hunt¹,²,*

Major histocompatibility complex-associated peptides have been considered as potential immunotherapeutic targets for many years. MHC class I phosphopeptides result from dysregulated cell signaling pathways that are common across cancers and both viral and bacterial infections. These antigens are recognized by central memory T cells from healthy donors, indicating that they are considered antigenic by the immune system and that they are presented across different individuals and diseases. Based on these responses and the similar dysregulation, phosphorylated antigens are promising candidates for prevention or treatment of different cancers as well as a number of other chronic diseases.

Nearly 30 years have passed since mass spectrometry was first used to understand the major histocompatibility complex (MHC) class I presentation process. After almost a year of trial and error to generate useful samples, the first MHC-associated peptide sequences were finally characterized by tandem mass spectrometry in 1992 (1). Since then, both instrument sensitivity and sample preparation techniques have improved significantly, allowing for identification of far more candidate peptides while decreasing analysis and validation times. Despite these advances, the promise of using peptides presented by the body’s own immune system as a widespread immunotherapy has encountered many challenges.

Mutated antigens have emerged at the forefront of these efforts (2–5). However, finding previously undiscovered mutations is difficult and generally requires prior knowledge from RNA sequencing (4–6). The difference in MHC expression between healthy and diseased cells is small, and neoantigens are expressed at much lower levels than canonical peptides (3–5, 7). Mutated and posttranslationally modified antigens were introduced concurrently and have similar reactivity (8), whereas modified antigens are comparatively understudied (9, 10). However, disease-associated modifications are caused by dysregulated signaling pathways that are common across different individuals, cancers, and even other diseases (10–12). Many cancer treatments already use these increased modification levels, particularly phosphorylation, for targeting cancerous cells over healthy ones, but immunotherapeutic development is largely focused on mutated antigens (10, 13–15). With these increases in cellular phosphorylation we see a similar increase in presentation of phosphorylated MHC peptides. Targeting these modified peptides should allow for development of immunotherapeutics that apply to an entire HLA type instead of requiring personalized treatments.

The first phosphorylated MHC I peptide identified by mass spectrometry was published in 1998 (8). That peptide (RVAsPTSGV) has since been found in 22 samples across nine types of cancer and two HLA types (A*02:01 and A*68:02) (Hunt Lab Phosphopeptide List (2020), unpublished data). An elongated version, RVAsPTSGVK, has been found in 16 samples, 4 cancers, and two HLA types (A*03:01 and A*11:01) (Hunt Lab Phosphopeptide List (2020), unpublished data). Since 1998, our laboratory has identified more than 2500 potential targets across 15 types of cancer; approximately 1000 of these have been found in multiple cancer types (Hunt Lab Phosphopeptide List (2020), unpublished data). (16) Some phosphopeptides have been found in up to 13 types of cancer and ~40 samples (Hunt Lab Phosphopeptide List (2020), unpublished data). Our current methodology for phosphopeptide enrichment and analysis can produce anywhere from tens to hundreds of MHC I phosphopeptide identifications using 100 to 500 mg of cancerous tissue (17, 18). Studies looking for mutated antigens use similar amounts of tissue and generally identify under 10 mutated antigens, most of which are specific to the tissue and patient (7, 19). Conversely, more than 50% of the phosphopeptide identifications for any given sample are generally already identified in another sample (depending on the prevalence of the HLA types) (Hunt Lab Phosphopeptide List (2020), unpublished data). Approximately 80% of phosphopeptides tested can generate central memory T cell responses from healthy donors (Hunt Lab Phosphopeptide List (2020), unpublished data). This response indicates that their immune system considers these peptides to be antigenic and that the response has overcome central tolerance (20, 21). Therefore, peptides...
MHC Phosphopeptides as Targets for Immunotherapeutics

that generate a healthy donor response are promising targets for immunotherapy of multiple cancers, as well as any other disease that causes their expression (20, 21).

Research indicates that many class I MHC phosphopepti-
dides are uniquely expressed on diseased cells and are po-
tential targets for the immunotherapy of hepatocellular cancer, breast cancer (22), melanoma (23), colorectal cancer (16), leukemias (21), and other cancers (24–27). Of particular note are results obtained in a recent preclinical trial that used two of our class I MHC phosphopeptides to treat high-risk melanomas (28). T cell responses to one peptide were observed in 5 of 12 patients. Another peptide generated T cell responses in 2 of 12 patients. Adverse effects were minimal. Two additional clinical trials that use multiple class I MHC phosphopeptides to treat acute myeloid leukemia and colorectal cancer are in the planning stages.

Two important questions are posed by these findings: (a) why do dysregulated cell signaling pathways generate the same phosphopeptide antigens on multiple types of cancer and (b) why do healthy blood donors, with no sign of auto-
immune disease, have central memory T cells that recognize and kill cells that present these same class I MHC phospho-
peptide antigens? Answers to both questions likely involve three major tumor suppressor proteins: protein phosphatase 2A (PP2A) (29–36), retinoblastoma protein (pRb) (37), and tumor suppressor protein 53 (p53) (38).

ONCOGENIC PROTEINS AND PHOSPHORYLATION

Phosphopeptide antigens are derived from dysregulated, cell signaling pathways common to many different cancers. In normal cells, phosphorylation is generally a brief event. However, dysregulation in cancerous cells extends the length of phosphorylation, allowing the proteasome sufficient time to degrade the phosphorylated protein forms and present phosphopeptide antigens on class I MHC mole-
cules. Since the same cell signaling pathways are also dysregulated during many viral and bacterial infections, many of the same phosphopeptide antigens should be presented. Therefore, these antigens could be employed for immunotherapy of, or vaccination against, both infectious agents and cancer.

PP2A is the most abundant serine/threonine phosphatase in the cell (comprising ~0.1%–1% of all cellular proteins) and plays a major role in the regulation of many important cell signaling pathways (e.g., Wnt, P13K/Akt, MAPK, and c-Myc) that control cell proliferation, transformation, and apoptosis (29–36). PP2A exists as a heterotrimeric complex consisting of a scaffolding subunit (A), regulatory subunit (B), and a catalytic subunit (C). There are two isoforms for subunit A, two isoforms for subunit C, and four classes of subunit B, each of which has 2 to 5 isoforms and additional splice variants. The regulatory subunits allow for cellular localization and impart specificity toward different substrates (29). As the most prolific and abundant phosphatase, altering the activity of PP2A should have the greatest effect on phosphorylation states (31, 32).

A similar phosphatase, protein phosphatase 1 (PP1), is the next most prolific phosphatase. PP1 lacks a scaffolding sub-
unit but has four isoforms of the catalytic subunit and about 60 known regulatory subunits (39, 40). The catalytic subunits of the two phosphatases have a high degree of sequence ho-
mology but have different substrate binding motifs (41). Both are also reversibly phosphorylated near the C terminus to control activation (42). Since the catalytic subunits are so similar, inhibitors that target the PP2A catalytic subunit or phosphate frequently have a significant effect on PP1 as well (34, 43).

PP1 and PP2A together are responsible for the vast majority of dephosphorylation in the cell, so dysregulation of one or both of them would have a significant effect on cellular phosphorylation (44, 45). Many phosphorylation sites may be dephosphorylated by multiple phosphatases, but a reduction in PP2A activity would cause a large reduction in overall phosphatase activity. Therefore, even if there are redundant phosphatases for a site, a large reduction in the total available phosphatase for a site should cause an extended phosphor-
phosphorylation event. Although kinases are also dysregulated in can-
cancer, each one is responsible for a far smaller subset of sites, meaning they individually have a much smaller effect on cellular phosphorylation. For example, all 15 members of the protein kinase C family, the most prolific kinase family, are together predicted to be responsible for ~20% of phosphoryl-
phosphorylation sites (46, 47). Based on their relative levels of control over phosphorylation, inhibition of PP2A and/or PP1 should increase cellular phosphorylation much more than over-
activation of any kinase or kinase family.

PP2A inhibition is accepted as a prerequisite for cellular transformation and is likely responsible for many of the phosphorylated antigens that we have observed across cancers (48–51). Accordingly, it has become a major area of research in the development of cancer therapeutics (13, 29, 52, 53). PP2A can be directly inactivated by somatic mutation and phosphorylation or demethylation near the C terminus of the catalytic subunit (29, 42). Somatic mutations are found in about 8% of cancer cases, with the scaffolding subunit as the most common site (20%) (29). Mutation of the scaffolding subunit generally causes an inability to bind regulatory sub-
units, thereby preventing the phosphatase from localizing or associating with its targets (29). Although these direct in-
hibitions are present in some cancers, PP2A is more commonly inactivated by upregulation of endogenous PP2A inhibitors: protein SET (also known as inhibitor 2 of PP2A) and cancerous inhibitor of phosphatase 2A (CIP2A). CIP2A, the more heavily studied of the two, is overexpressed in ~40% to 90% of patients across different cancer types (54–58). Both CIP2A and SET appear to inhibit PP2A activity by binding to the catalytic subunit, preventing it from associating with the rest of the holoenzyme (59–61). High CIP2A and/or SET levels
oncogenic protein, is a sequence-specific DNA-binding protein that regulates transcription and promotes cell cycle arrest and apoptosis (38, 65). It can activate DNA repair proteins when DNA has sustained damage, arrest growth by holding the cell cycle at the G1/S regulation point to give the repair machinery time to fix DNA sequence errors, and initiate programmed cell death (apoptosis) if the damage proves to be irreparable. p53 is activated by phosphorylation and degraded in the proteasome when it is ubiquitinated by the ubiquitin ligase Mdm2 (66). PP2A is responsible for dephosphorylation by PP2A, reactivation becomes very difficult with even a small amount of dysregulation.

Tumor suppressor p53, perhaps the most heavily studied oncogenic protein, is a sequence-specific DNA-binding protein that regulates transcription and promotes cell cycle arrest and apoptosis (38, 65). It can activate DNA repair proteins when DNA has sustained damage, arrest growth by holding the cell cycle at the G1/S regulation point to give the repair machinery time to fix DNA sequence errors, and initiate programmed cell death (apoptosis) if the damage proves to be irreparable. p53 is activated by phosphorylation and degraded in the proteasome when it is ubiquitinated by the ubiquitin ligase Mdm2 (66). PP2A is responsible for dephosphorylation at Thr55 that activates p53 and for a number of phosphorylation sites on Mdm2 that allow for appropriate p53 phosphorylation as well as phosphoantigen presentation. Therefore, disruption that results in dephosphorylation of Mdm2 should lead to the presentation of phosphorylated MHC I peptides. Since inhibition of these proteins should lead to extended protein phosphorylation through phosphatase inhibition, it should also lead to the presentation of phosphorylated MHC I peptides.

DISEASE-ASSOCIATED PP2A INHIBITION

Since p53, pRb, and PP2A are important in pathways that are responsible for the success of any disease (proliferation and avoidance of immune detection), they are also common targets for some of the most chronic and difficult-to-treat diseases, regardless of the pathogen (36, 39, 65). If this reasoning is valid, any disease that targets any combination of p53, pRb, or PP2A has the potential to produce the same MHC-associated phosphopeptides as cancerous cells (Fig. 2). In other words, an immunotherapy that utilizes MHC-associated phosphopeptides should work against diseases that targets any of these three proteins. In this section, we will discuss several diseases that target p53, pRb, and/or PP2A. Since inhibition of these proteins should lead to extended protein phosphorylation through phosphatase inhibition, it should also lead to the presentation of phosphorylated MHC I peptides.

Hepatitis B and C Viruses

Worldwide, there are 140 million people infected with hepatitis C virus (HCV) and more than 250 million people with hepatitis B virus (HBV) (70). Both viruses can cause hepatocellular cancer. HCV consists of a single-stranded RNA (9600 nucleotide bases) surrounded by a protected shell of proteins. The viral RNA codes for a single polyprotein (~3000 aa) that is posttranslationally cleaved into two highly glycosylated structural proteins E1 and E2, a transmembrane protein p7, and six nonstructural, accessory proteins: NS2, NS3, NS4A, NS4B, NS5A, and NS5B. HCV does not integrate its genome into the host chromosomal DNA (70). It exhibits a high mutational rate and deregulates many host cellular processes. An accessory protein, NS5B, forms a complex with the retinoblastoma tumor suppressor protein (pRb) that is then targeted for degradation in the proteasome following ubiquitination by human papillomavirus (HPV) E1 and E2.
around tumors is generally compromised in some way, either because the margin and tumor were not cleanly separated or because the tissue surrounding the tumor suffers from the same conditions that caused the tumor (e.g., alcoholic liver disease). In a study comparing phosphorylation between HCC cases, margin tissue from five patients without these diagnoses (caused by adenoma transformation) (Fig. 3, C and D) or unknown etiology (Fig. 3, A and B) expressed, on average, 11% (0%–25%) of the phosphorylated MHC peptides seen on the corresponding tumor tissue (N. Buettner et al., unpublished data). In contrast, margin tissue from five patients with HBV and/or HCV shows far more phosphorylation, ranging from 55% to 675% of the corresponding tumor’s expression (Fig. 3, E–H) (N. Buettner et al., unpublished data). This supports the theory that hepatitis infection causes increased phosphopeptide expression prior to transformation.

**Human Papillomavirus**

Human papillomavirus (HPV) infects the basal cells of human epithelia and is the main causative agent for many human tumors including cervical, anal, and oral cancers (77, 78). There are close to 200 different HPV types, many of which are oncogenic. The oncogenic strains are also referred to as “high risk” and produce the same proteins as the low-risk variants, but with sequence variations that alter activity in a way that can lead to cellular transformation (79, 80). Most notably, HPV-16 and HPV-18 are responsible for the majority of cases of cervical cancer, the second most common cancer in women worldwide (78). Cells infected with high-risk HPV are often able to carry out transformation without cell lysis or inflammation, allowing it to avoid detection by the immune system (78).

Oncogenic variants of HPV encode a 98-residue phosphoprotein (E7) that binds to the active, unphosphorylated form of pRb as well as related proteins p130 and p107 and targets them for degradation in the proteasome (80, 81). When the E7 protein targets pRb for degradation, E2F1, a member of the E2F1-3 transcription factor family that was repressed by pRb, now becomes activated and upregulates expression of the protein CIP2A (50, 82, 83). The E7 protein produced by low-risk strains of the virus is less phosphorylated than the high-risk variants and has significantly lower affinity for pRb (79, 84). Another HPV accessory protein, E6, upregulates the DNA cytosine deaminase APOBEC-3B (A3B), an enzyme that converts cytosine to uracil and causes hypermutation of the viral DNA (80, 85, 86). Normally, this would activate the tumor suppressor protein p53, to trigger apoptosis. Unfortunately, the 158-residue E6 protein and the E6AP cellular protein form a complex that allows them to bind p53 and target it for ubiquitination and degradation in the proteasome (80). The low-risk variant of E6 is still able to associate with p53 but is unable to target it for degradation (67).

**Epstein Barr Virus**

More than 90% of adults in the world have been infected with the Epstein Barr virus (EBV) (also known as human...
herpesvirus 4), and most continue to have a lifelong dormant infection (88). EBV infects both B cells and epithelial cells. The reservoir for the latent virus is primarily resting, central memory B cells. EBV is known to cause infectious mononucleosis and a variety of cancers such as Hodgkin’s lymphoma, Burkitt’s lymphoma, gastric cancer, and nasopharyngeal carcinomas (89, 90).

The virus is composed of a double-stranded DNA helix that codes for 85 proteins and is surrounded by a protein nucleocapsid and an envelope of both lipids and glycoproteins. Regulatory proteins of note include six nuclear antigens (EBNA-1, -2, -3A, -3B, 3C, and the EBV nuclear antigen-leader protein, EBNA-LP) plus three EBV latent membrane proteins (LMP-1, -2A, and -2B) (91). EBNA-3C (also known as EBNA-6) binds the mitochondrial ribosomal protein MRPS18-2 and targets it to the nucleus, where it binds to the pRb and liberates the E2F1 group of transcription factors (92). EBNA-3C can also recruit the SCFSkp2 ubiquitin ligase complex that mediates ubiquitination and degradation of pRb, resulting in high levels of transcription (93). EBNA-3C also enhances the intrinsic ubiquitin ligase activity of Mdm2 toward p53, which in turn facilitates p53 ubiquitination and degradation (94). As with the other viruses, this should liberate the transcription factors that upregulate expression of endogenous PP2A protein inhibitors SET and CIP2A. In addition, a truncated form of EBNA-LP has been shown to interact with and bind to the catalytic subunit of PP2A (95).

**Human Immunodeficiency Virus**

The human immunodeficiency virus (HIV-1) is a retrovirus that infects CD4+ T cells, macrophages, and dendritic cells and eventually causes AIDS. More than 40 million people worldwide are infected with the virus. HIV-1 is composed of two copies of single-stranded RNA that produce 16 proteins (96). Four HIV accessory proteins, Vif, Vpr, Nef, and Vpu, share the ability to target cellular proteins for proteasomal degradation and are essential for pathogenesis in vivo (96, 97). Recently, the accessory protein Vif was discovered to be necessary and sufficient for culin-5 dependent ubiquitination and proteasomal degradation of all members of the B56 family of regulatory subunits for PP2A (69, 98). Inhibition of PP2A by Vif produced hyperphosphorylation of cellular proteins that mirrored previously reported changes seen when PP2A was treated with the small molecule inhibitor okadaic acid in transformed cells (98, 99).

**Coronaviruses**

Severe acute respiratory syndrome associated coronaviruses (SARS-CoVs) are highly infectious single-stranded RNA viruses that cause respiratory illness. SARS-CoV-1 was the first pandemic of the 21st century, infecting approximately 8000 people from 29 countries between 2002 and 2004. A similar but far more infectious virus emerged in 2019, the novel coronavirus SARS-CoV-2. Infected over 70 million people worldwide in 2020, SARS-CoV-2 has become a far worse pandemic. Since SARS-CoV-2 is relatively novel, there is little published research regarding its cell dysregulation and long-term effects. However, there are several papers that show PP2A or pRb dysregulation in SARS-CoV-1 infected cells, and studies linking them with SARS-CoV-2 are beginning to emerge.

SARS-CoV-1 and SARS-CoV-2 encode 28 and 26 proteins, respectively (100). Nonstructural protein 15 (Nsp15) is a 180-
residue protein that has 89% sequence homology between SARS-CoV-1 and SARS-CoV-2 (100). Nsp15 from SARS-CoV-1 was previously found to target pRb for degradation through binding with a LXCXE/D motif (101). This is the same motif that the HPV E7 protein uses for targeting pRb (102, 103). This motif is conserved in SARS-CoV-2 (L331-D335 in both proteins) and should serve the same function. Another SARS-CoV-1 protein, ORF7a, was also found to inhibit pRb through hyperphosphorylation (104). In addition, the scaffolding subunit of PP2A was found to be three times less abundant in cells infected with either SARS-CoV, although the cause is undetermined (105). New research indicates that the SARS-CoV-2 spike glycoprotein contains motifs for binding to the B56α subunit, which would cause further inhibition of PP2A (106).

**Helicobacter pylori**

*Helicobacter pylori* is a gastrointestinal bacterium that is present in about 50% of the population. In 2005, the Nobel prize in physiology or medicine was awarded for discovery of its link with gastritis and peptic ulcer disease. Certain strains of the bacteria are known to release the protein cytotoxin-associated antigen A (CagA). CagA-positive strains have a stronger link with development of peptic ulcers and are associated antigen A (CagA). CagA has also been shown to increase CIP2A expression, which is likely caused by the pRb inactivation (109).

**Fusobacterium nucleatum**

*Fusobacterium nucleatum* is a gram-negative anaerobe that is usually the most abundant bacterium in the oral cavity and plays a key role in the development of dental plaque and a number of oral diseases (110). However, it also flourishes outside the oral cavity and is responsible for many infections. Presence of this bacteria has been strongly connected to colorectal carcinogenesis and progression (111–114). It has also been linked to esophageal (115), intestinal (116), and breast (117) cancers. It is known to promote colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling (111, 114). The *F. nucleatum* genome codes for the protein FadA, which binds to E-cadherin on colorectal cells and mediates attachment and invasion of the bacterium. Both FadA (114) and the *F. nucleatum* lipopolysaccharide (118) have been reported to activate β-catenin signaling, which should result in generation of CIP2A.

**Neurodegenerative Diseases**

Abnormal hyperphosphorylation of protein Tau is the main hallmark of a number of neurodegenerative disorders called tauopathies. The most studied of these is Alzheimer’s disease (AD), but they include a number of other dementias and neurodegeneration following traumatic brain injury or encephalopathy (119). In these diseases, Tau is 3 to 4 times more phosphorylated than it is in healthy cells. This hyperphosphorylation leads to microtubule disorganization, protein aggregation, and cell death (120). PP2A is responsible for 70% of phosphatase activity for protein Tau and has become a major topic of study in the field (121). Inhibition of PP2A using okadaic acid has been shown to induce p-Tau formation as well as other characteristics of tauopathies including cognitive impairment, protein aggregation, and cell death (120). Increased pRb phosphorylation and decreased PP2A methylation have been observed in AD, both of which should ultimately cause PP2A inhibition (33). Increased levels of both CIP2A and SET colocalized with increased p-Tau levels in stains of AD tissue (122).

Immunopeptidomic studies of brain tissue are difficult and are not frequently attempted (123). Most obviously, the brain is not a common operation site and is unlikely to be donated. In addition, brain tissue has low MHC expression, about 70 times lower than most other tissue types (124). However, we were able to identify two phosphorylated MHC I peptides in a 400-mg cadaverous neurodegenerative sample. Both were previously found in multiple cancers and generate responses from healthy donor memory T cells. We have not identified any phosphorylated antigens on cadaverous tissues from healthy donors.

**CONCLUSIONS**

From our studies over the past 20 years, we have observed that phosphorylated MHC peptides are expressed primarily on diseased tissue and across multiple cancer types. Since PP2A is prolific and its inhibition is heavily linked with cancer, it is the most likely cause of the increased phosphorylated antigen expression seen in cancer tissues. If this increase in phosphopeptide antigens is caused by PP2A dysregulation, then a treatment developed using these phosphopeptides could apply to any disease with the same dysregulation. Expression of phosphorylated MHC antigens by cells infected with these pathogens would explain why healthy donors with no prior cancer diagnosis have memory T cells that recognize these antigens. That is, common viruses such as EBV may give the immune system an opportunity to recognize these peptides as antigenic, resulting in memory T cells.

Even though the limited evidence we have managed to collect thus far is promising, far more research is needed to confirm our theories. Generating the quantities of cells needed to confirm whether other diseases cause expression of cancer-linked phosphorylated antigens is difficult. Although infection of cancerous cell lines is commonplace, efficient infection of high quantities of healthy cells is difficult. In addition, confirmation would require cells that are infected but not cancerous. Since many of these viruses are oncogenic, the line between infection and cancer may be uncertain. For
example, EBV is frequently used to immortalize cells, so it is difficult to define when to consider the cells transformed rather than merely infected.

Despite these challenges, modified antigens are appealing targets for immunotherapeutic treatments for many reasons. Not only are they expressed across many different cancers and individuals, but they also have the potential to allow treatment of many other chronic and debilitating diseases. Since this is a less common approach for antigen identification, there is ample space for discovery of novel antigens or diseases. The concept could be further expanded to study other posttranslational modifications, such as methylation and glycosylation, which are also dysregulated across different cancers and diseases.

Acknowledgments—We would like to acknowledge all the scientists who have contributed to this research over the past 30 years. We also thank Maria C. Panepinto for comments on the manuscript.

Funding and additional information—D. F. H. acknowledges NIH AI033993 and GM037537 for years of support. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Author contributions—K. E. M., J. S., and D. F. H. are responsible for all aspects of this article.

Conflict of interest—J. S. and D. F. H. have financial interest in Agenus, Inc.

Abbreviations—The abbreviations used are: AD, Alzheimer’s disease; CagA, cytotoxin-associated antigen A; CIP2A, cancerous inhibitor of phosphatase 2A; E2F, E2 family transcription factor; EBV, Epstein Barr virus; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HPV, human papillomavirus; MHC, major histocompatibility complex; p53, protein 53; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; pRb, retinoblastoma protein; SARS-CoV, severe acute respiratory syndrome associated coronavirus.

Received January 23, 2021, and in revised form, May 11, 2021

Published: MCPRO Papers in Press, June 12, 2021, https://doi.org/10.1016/j.mcpromo.2021.100112

REFERENCES

1. Hunt, D. F., Henderson, R. A., Shabanowitz, J., Sakaguchi, K., Michel, H., Sevill, N., Cox, A. L., Appella, E., and Engelhard, V. H. (1992) Charac-
terization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry. Science 256, 1261–1263

2. Castle, J. C., Uduman, M., Pabla, S., Stein, R. B., and Buell, J. S. (2019) Mutation-derived neoantigens for cancer immunotherapy. Front. Immunol. 10, 1856

3. Schumacher, T. N., Scheper, W., and Kvistborg, P. (2019) Cancer neo-
antigens. Annu. Rev. Immunol. 37, 173–200

4. Jiang, T., Shi, T., Zhang, H., Hu, J., Song, Y., Wei, J., Ren, S., and Zhou, C. (2019) Tumor neoantigens: From basic research to clinical applications. J. Hematol. Oncol. 12, 1–13

5. Han, X. J., Ma, X. L., Yang, L., Wei, Y. Q., Peng, Y., and Wei, X. W. (2020) Progress in neoantigen targeted cancer immunotherapies. Front. Cell Dev. Biol. 8, 728

6. Sahin, U., Derhanvanessian, E., Miller, M., Kloke, B. P., Simon, P., Löwer, M., Bukur, V., Tadmor, A. D., Luxemburger, U., Schrörs, B., Ormoko, T., Vonmehr, M., Albrecht, C., Parczynski, A., Kuhn, A. N., et al. (2017) Personalized RNA mutanome vaccines mobilize poly-specific thera-
pic immunity against cancer. Nature 547, 222–226

7. Kote, S., Pirog, A., Bedran, G., Alfaro, J., and Dapic, I. (2020) Mass spectrometry-based identification of MHC-associated peptides. Cancers (Basel) 12, 535

8. Hogan, K. T., Esising, D. P., Cupp, S. B., Lekstrom, K. J., Deacon, D. D., Shabanowitz, J., Hunt, D. F., Engelhard, V. H., Slingluff, C. L., and Ross, M. M. (1998) The peptide recognized by HLA-A68.2-restricted, squa-
mous cell carcinoma of the lung-specific cytotoxic T lymphocytes is derived from a mutated elongation factor 2 gene. Cancer Res. 58, 5144–5150

9. Solleder, M., Guillame, P., Racile, J., Michaux, J., Pak, H. S., Müller, M., Coukos, G., Bassani-Sternberg, M., and Geliller, D. (2020) Mass spec-
trometry based immunopeptidomics leads to robust predictions of phosphorylated HLA class I ligands. Mol. Cell Proteomics 19, 390–404

10. Zeneyedpour, L., Stan-van T Hoff, J., and Luider, T. (2020) Using phos-
phoproteomics and next generation sequencing to discover novel therapeutic targets in patient antibodies. Expert Rev. Proteomics 17, 675–684

11. Sever, R., and Brugge, J. S. (2015) Signal transduction in cancer. Cold Spring Harb. Perspect. Med. 5, a006098

12. Berg, J. M., Tymoczko, J. L., and Stryer, L. (2007). In: Biochemistry, 6th Ed, W.H. Freeman, New York, NY: 400–405

13. Mazhar, S., Taylor, S. E., Sangodkar, J., and Narla, G. (2019) Targeting PP2A in cancer: Combination therapies. Biochem. Biophys. Acta. Mol. Cell Res. 1866, 51–63

14. Ardito, F., Giuliani, M., Perrone, D., Troiano, G., and Muzio, L. (2017) The crucial role of protein phosphorylation in cell signaling and its use as targeted therapy (review). Int. J. Mol. Med. 40, 271–280

15. Zhang, Q., and Claret, F. X. (2012) Phosphoproteomes: The new brakes for cancer development? Enzyme Res. 2012, 659649

16. Hunt, D. F., Shabanowitz, J., Myers, P. T., Cobbold, M., Böttner, N., Malaker, S. A., and Penny, S. (2019) Target peptides for cancer therapy and diagnostics. US patent application 16/098,634

17. Heather, J. M., Myers, P. T., Shi, F., Aziz-Zanjani, M. O., Mahoney, K. E., Perez, M., Morin, B., Brittsan, C., Shabanowitz, J., and Hunt, D. F. (2019) Murine xenograft bioreactors for human immunopeptide discovery. Sci. Rep. 9, 1–15

18. Abelin, J. G., Trantham, P. D., Penny, S. A., Patterson, A. M., Ward, S. T., Hildebrand, W. H., Cobbold, M., Bai, D. L., Shabanowitz, J., and Hunt, D. F. (2015) Complementary IMAC enrichment methods for HLA-
associated phosphopeptide identification by mass spectrometry. Nat. Protoc. 10, 1308–1318

19. Bassani-Sternberg, M., Bräunlein, E., Klar, R., Engeltner, T., Sintycyn, P., Audehnn, S., Straub, M., Weber, J., Sottala-Huspenina, S., Specht, K., Martignoni, M. E., Werner, A., Hein, R., Busch, D. H., Peschel, C., et al. (2016) Direct identification of clinically relevant neopeptides presented on native human melanoma tissue by mass spectrometry. Nature. Commun. 7, 1–16

20. Henderson, R. A., Cox, A. L., Sakaguchi, K., Appella, E., Shabanowitz, J., Hunt, D. F., and Engelhard, V. H. (1993) Direct identification of an endogenous peptide recognized by multiple HLA-A2.1-specific cytotoxic T cells. Proc. Natl. Acad. Sci. U. S. A. 90, 10275–10279

21. Cobbold, M., De La Peña, H., Norris, A., Polefrone, J. M., Qian, J., English, A. M., Cummings, K. L., Penny, S., Turner, J. E., Cottinge, J., Abelin, J. G., Malaker, S. A., Zarling, A. L., Huang, H.-W., Goodyear, O., et al. (2013) MHC class I-associated phosphopeptides are the targets of memory-
lke immunity in leukemia. Sci. Translat. Med. 5, 203ra125

22. Hunt, D. F., Norris, A., English, A. M., Shabanowitz, J., Hildebrand, W. H., and Hawkins, O. E. (2020) Identification of MHC class I phospho-peptide antigens from breast cancer utilizing SHLA technology and comple-
mentary enrichment strategies. US patent 10,535

23. Topalian, S. L., Deportieu, F. A., Hunt, D. F., Shabanowitz, J., Qian, J., Engelhard, V. H., and Zarling, A. L. (2016) Peptidephosphopeptides as mela-
noma vaccines. US patent 9,279,011
MHC Phosphopeptides as Targets for Immunotherapeutics

24. Zarling, A. L., Polefrone, J. M., Evans, A. M., Mikesk, L. M., Shabanowitz, J., Lewis, S. T., Engelhard, V. H., and Hunt, D. F. (2006) Identification of class I MHC-associated phosphopeptides as targets for cancer immunotherapy. *Proc. Natl. Acad. Sci. U. S. A.* 103, 14889–14894

25. Mohammed, F., Cobbold, M., Zarling, A. L., Salim, M., Barrett-Wilt, G. A., Shabanowitz, J. H., Hunt, D. F., Engelhard, V. H., and Willcox, B. E. (2008) Phosphorylation-dependent interaction between antigenic peptides and MHC class I: a molecular basis for the presentation of transformed self. *Nat. Immunol.* 9, 1236–1243

26. Mohammed, F., Stones, D. H., Zarling, A. L., Willcox, C. R., Shabanowitz, J., Cummings, K. L., Hunt, D. F., Cobbold, M., Engelhard, V. H., and Willcox, B. E. (2017) The antigenic identity of human class I MHC phosphopeptides is critically dependent upon phosphorylation status. *Onctarget* 8, 54160–54172

27. Hunt, D. F., Shabanowitz, J., Cottine, J., English, A. M., Norris, A., Engelhard, V. H., Cobbold, M., Cummings, K. L., Zarling, A., and Obeng, R. C. (2013) Class I MHC phosphopeptides for cancer immunotherapy and diagnosis. US patent application 13/699,563

28. Engelhard, V. H., Obeng, R. C., Cummings, K. L., Petroni, G. R., Ambikahutwala, A. L., Chianese-Bullock, K. A., Smith, K. T., Lulu, A., Varhegyi, N., Smolkin, M. E., Myers, P., Mahoney, K. E., Shabanowitz, J., Buetterner, N., Hall, E. H., et al. (2020) MHC-restricted phosphopeptide antigens: Preclinical validation and first-in-humans clinical trial participants in patients with high-risk melanoma. *J. Immunother. Cancer* 8, e003262

29. Sangodkar, J., Farrington, C. C., McClinch, K., Galsky, M. D., Kastrinsky, J., Janssens, V., Longin, S., and Goris, J. (2008) PP2A holoenzyme assembly: A biological strategy from DNA/RNA tumor viruses to HIV-1. *Trends Biochem. Sci.* 33, 14894–14895

30. Li, T., Du, P., and Xu, N. (2010) Identifying human kinase-specific protein phosphorylation sites by integrating heterogeneous information from various sources. *PLoS One* 5, e15411

31. Janssens, V., Goris, J., and Van Hoof, C. (2005) PP2A: The expected tumor suppressor. *Curr. Opin. Genet. Dev.* 15, 34–41

32. Rangarajan, A., Hong, S. J., Gifford, A., and Weinberg, R. A. (2004) Species- and cell-type-specific requirements for cellular transformation. *Cancer Cell* 6, 171–183

33. Wang, X., Gao, P., Wang, M., Liu, J., Lin, J., Zhang, S., Zhao, Y., Zhang, J., Pan, W., Sun, Z., Sun, F., Zhao, W., Guo, C., and Wang, Q. (2017) Feedback between EZF1 and CIP2A regulated by human papillomavirus E7 in cervical cancer: Implications for prognosis. *Am. J. Transl. Res.* 9, 2337–2339

34. Hahn, W. C., Counter, C. M., Lukbert, A. S., Beijersbergen, R. L., Brooks, M. W., and Weinberg, R. A. (1999) Creation of human tumour cells with defined genetic elements. *Nature* 400, 464–468

35. Kauko, O., Imanishi, S. Y., Kuleskii, E., Yetukuri, L., Lajala, T. D., Sharma, M., Pavic, K., Aakula, A., Rupp, C., and Junpanen, M. (2020) Phosphoproteome and drug-response effects mediated by the three protein phosphatase 2A inhibitor proteins CIP2A, SET, and PME-1. *J. Biol. Chem.* 295, 4194–4211

36. Chung, V., Mansfield, A. S., Brathe, F., Richards, D., Durivage, H., Ungerleider, R. S., Johnson, F., and Kovach, J. S. (2017) Safety, tolerability, and preliminary activity of LB-100, an inhibitor of protein phosphatase 2A, in patients with relapsed solid tumors: An open-label, dose escalation, first-in-human, phase I trial. *Clin. Cancer Res.* 23, 3277–3284

37. Liu, J., Wang, X., Zhou, G., Wang, H., Xiang, L., Cheng, Y., Liu, W., Wang, Y., Jia, J., and Zhao, W. (2011) Cancerous inhibitor of protein phosphatase 2A is overexpressed in cervical cancer and upregulated by human papillomavirus 16 E7 oncoprotein. *Gynecol. Oncol.* 122, 430–436

38. Bocekman, C., Lassus, H., Hemmes, A., Lemenin, A., Westermarck, J., Haglund, C., Bubzow, R., and Ristimaki, A. (2011) Prognostic role of CIP2A expression in serous ovarian cancer. *Br. J. Cancer* 105, 899–905

39. Côte, C., Laine, A., Chanrion, M., Edgren, H., Mattila, E., Liu, X., Jonkers, J., Ivaska, J., Isola, J., Darbon, J. M., Kallioniemi, O., Thesis, S., and Westermarck, J. (2009) CIP2A is associated with human breast cancer aggressivity. *Clin. Cancer Res.* 15, 5092–5100

40. Dong, Q. Z., Wang, Y., Dong, X. J., Li, Z. X., Cui, Q. Z., and Wang, E. H. (2011) CIP2A is overexpressed in non-small cell lung cancer and regulates epithelial-mesenchymal transition. *Am. J. Surg. Oncol.* 18, 857–865

41. Lucas, C. M., Harris, R. J., Giannoudis, A., Copland, M., Slupsky, J. R., and Clark, R. E. (2011) Cancerous inhibitor of PP2A (CIP2A) at diagnosis of chronic myeloid leukemia is a critical determinant of disease progression. *Blood* 117, 6660–6668

42. Junittia, M. R., Puustinen, P., Niemelä, M., Ahola, R., Arnold, H., Böttzaw, T., Ala-aho, R., Nielsen, C., Ivaska, J., Taya, Y., Lu, S. L., Lin, S., Chan, E. K. L., Wang, X. J., Grünman, R., et al. (2007) CIP2A inhibits PP2A in human malignancies. *Clin. Exp. Pharmacol. Physiol.* 34, 510–521

43. Li, M., Makkinje, A., and Damuni, Z. (1996) The myeloid leukemia-associated protein SET is a potent inhibitor of protein phosphatase 2A. *J. Biol. Chem.* 271, 11059–11062

44. Li, M., Makkinje, A., and Damuni, Z. (1996) Molecular identification of I1PP2A, a novel potent heat-stable inhibitor protein of protein phosphatase 2A. *Biochemistry* 35, 885–895

45. Cristóbal, I., Rincón, R., Manso, R., Caramés, C., Zazo, S., Madoz-Gúrpide, J., Rojo, F., and García-Foncillas, J. (2015) Deregulation of the PP2A inhibitor SET shows promising therapeutic implications and determines poor clinical outcome in patients with metastatic colorectal cancer. *Clin. Cancer Res.* 21, 347–356

46. Kudnison, A. G. (1971) Mutation and cancer: Statistical study of retinoblastoma. *Proc. Natl. Acad. Sci. U. S. A.* 68, 820–823

47. Hickman, E. S., Moroni, M. C., and Helin, K. (2002) The role of p53 and pRB in apoptosis and cancer. *Curr. Opin. Genet. Dev.* 12, 60–66
89. Shannon-Lowe, C., Rickinson, A. B., and Bell, A. I. (2017) Epstein-barr virus infection and nasopharyngeal carcinoma. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **372**, 20160270

90. Tsao, S. W., Tsang, C. M., and Lo, K. W. (2017) Epstein-barr virus infection and nasopharyngeal carcinoma. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **372**, 20160270

91. Grywalska, E., and Rolinski, J. (2015) Epstein-Barr virus-associated lymphomas. *Semin. Oncol.* **42**, 291–303

92. Kashiwakura, E., Yurchenko, M., Yenamandra, S. P., Snopok, B., Isaguliants, M., Szekely, L., and Klein, G. (2008) EBV-encoded EBNA-6 binds and targets MRS18-2 to the nucleus, resulting in the disruption of pRb-E2F1 complexes. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 5489–5494

93. Knight, J. S., Sharma, N., and Robertson, E. S. (2005) Epstein-Barr virus latent antigen 3C can mediate the degradation of the retinoblastoma protein through an SCF cellular ubiquitin ligase. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 18562–18566

94. Saha, A., Murakami, M., Kumar, P., Bajaj, B., Sims, K., and Robertson, E. S. (2009) Epstein-Barr virus nuclear antigen 3C augments Mdm2-mediated p53 ubiquitination and degradation by deubiquitinating Mdm2. *J. Virol.* **83**, 4652–4669

95. Garibal, J., Hollville, E., Bell, A. I., Kelly, G. L., Renouf, B., Kawaguchi, Y., Rickinson, A. B., and Wiels, J. (2007) Truncated form of the Epstein-Barr virus protein EBNA-LP protects against caspase-dependent apoptosis by inhibiting protein phosphatase 2A. *J. Virol.* **81**, 7598–7607

96. Li, G., and De Clercq, E. (2016) HIV genome-wide protein associations: A review of 30 years of research. *Microbiol. Mol. Biol. Rev.* **80**, 679–731

97. Sugden, S. M., Bego, M. G., Thom, T. N. Q., and Cohen, E. A. (2016) Remodeling of the host cell plasma membrane by HIV-1 Nef and Vpu: A strategy to ensure viral fitness and persistence. *Viruses* **8**, 67

98. Greenwood, E. J. D., Matheson, N. J., Wals, K., van den Boomen, D. J. H., Antrobus, R., Williamson, J. C., and Lehner, P. J. (2016) Temporal proteomic analysis of HIV infection reveals remodelling of the host phosphoproteome by lentiviral Vif variants. *Elife* **5**, 1–30

99. Kauko, O., Lajasa, T. D., Jumppanen, M., Hintsanen, P., Suni, V., Hapaniemi, P., Corhals, G., Alttokatto, T., Westermarck, J., and Imanishi, S. Y. (2015) Label-free quantitative phosphoproteomics with novel pairwise abundance normalization reveals synergistic RAS and CIP2A signaling. *Sci. Rep.* **5**, 13099

100. Xu, J., Zhao, S., Teng, T., Abdalla, A. E., Zhu, W., Xie, L., Wang, Y., and Guo, X. (2020) Systematic comparison of two animal-to-human transmitted human coronaviruses: SARS-CoV-2 and SARS-CoV. *Viruses* **12**, 244

101. Bhardwaj, K., Liu, P., Leibowitz, J. L., and Kao, C. C. (2012) The corona-virus endoribonuclease Nsp15 interacts with retinoblastoma tumor suppressor protein. *J. Virol.* **86**, 4294–4304

102. Boyer, S. N., Wazer, D. E., and Band, V. (1996) E7 protein of human papilloma virus-16 induces degradation of retinoblastoma protein through the ubiquitin-proteasome pathway. *Cancer Res.* **56**, 4620–4624

103. Müller, N., Howley, P. M., Münker, M., and Harlow, E. (1989) The human papilloma-virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* **243**, 934–937

104. Yuan, X., Wu, J., Shan, Y., Yao, Z., Dong, B., Chen, B., Zhao, Z., Wang, S., Chen, J., and Cong, Y. (2006) SARS coronavirus 7a protein blocks cell cycle progression at G0/G1 phase via the cyclin D3/pp2b pathway. *Viruses* **8**, 47–85

105. Jiang, X., Yang, X., Wang, Z., Li, J., Zou, J., Chen, C., and Zeng, R. (2005) Quantitative analysis of severe acute respiratory syndrome (SARS)-associated coronavirus-infected cells using proteomic approaches: Implications for cellular responses to virus infection. *Mol. Cell. Proteomics* **4**, 902–913

106. [preprint] Maaraufi, H. (2020) LxxElE-like motif in spike protein of SARS-CoV-2 that is known to recruit the host PP2A-B56 phosphatase is a mimetic of artemisinin, an immunomodulator, of Brazilian green propolis. *bioRxiv*. https://doi.org/10.1101/2020.04.01.020941

107. Ding, S. Z., Goldberg, J. B., and Hatakeyama, M. (2010) Helicobacter pylori infection, oncogenic pathways and epigenetic mechanisms in gastric carcinogenesis. *Future Oncol.* **6**, 851–862

108. De Luca, A., Baidi, A., Russo, P., Todisco, A., Altucci, L., Giardullo, N., Pasquale, L., Iaquinto, S., D’Onofrio, V., Parodi, M. C., Maccio, G. G., and Iaquinto, G. (2003) Coexpression of Helicobacter pylori’s proteins CagA and HspB induces cell proliferation in AGS gastric epithelial cells, independently from the bacterial infection. *Cell. Res.* **63**, 6530–6536

109. Zhao, D., Liu, Z., Ding, J., Li, W., Sun, Y., Yu, H., Zhou, Y., Zeng, J., Chen, C., and Jia, J. (2013) Helicobacter pylori CagA upregulation of CIP2A is dependent on the Src and MEK/ERK pathways. *J. Med. Microbiol.* **59**, 259–265
110. Gagnaire, A., Nadel, B., Raoult, D., Neefjes, J., and Gorvel, J. P. (2017) Collateral damage: Insights into bacterial mechanisms that predispose host cells to cancer. *Nat. Rev. Microbiol.* 15, 1–20

111. Zhang, S., Cai, S., and Ma, Y. (2018) Association between Fusobacterium nucleatum and colorectal cancer: Progress and future directions. *J. Cancer* 9, 1652–1659

112. Bullman, S., Pedamallu, C. S., Sicinska, E., Clancy, T. E., Zhang, X., Cai, D., Neuberg, D., Huang, K., Guevara, F., Nelson, T., Chipashvili, O., Hagan, T., Walker, M., Ramachandran, A., Diosdado, B., et al. (2017) Analysis of Fusobacterium persistence and antibiotic response in colorectal cancer. *Science* 358, 1443–1448

113. Lee, S. A., Liu, F., Riordan, S. M., Lee, C. S., and Zhang, L. (2019) Global investigations of fusobacterium nucleatum in human colorectal cancer. *Front. Oncol.* 9, 566

114. Rubinstein, M. R., Wang, X., Liu, W., Hao, Y., Cai, G., and Han, Y. W. (2013) Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. *Cell Host Microbe* 14, 195–206

115. Yamamura, K., Baba, Y., Nakagawa, S., Mima, K., Miyake, K., Nakamura, K., Sawayama, H., Kinoshita, K., Ishimoto, T., Iwatsuki, M., Sakamoto, Y., Yamashita, Y., Yoshida, N., Watanabe, M., and Baba, H. (2016) Human microbiome Fusobacterium nucleatum in esophageal cancer tissue is associated with prognosis. *Clin. Cancer Res.* 22, 5574–5581

116. Kostic, A. D., Chun, E., Robertson, L., Glickman, J. N., Gallini, C. A., Michaud, M., Clancy, T. E., Chung, D. C., Lochhead, P., Hold, G. L., El-Omar, E. M., Brenner, D., Fuchs, C. S., Meyerson, M., and Garrett, W. S. (2013) Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 14, 207–215

117. Parhi, L., Alon-Maimon, T., Sol, A., Nejman, D., Shhadeh, A., Fainsod-Levi, T., Yajuk, O., Isaacson, B., Abed, J., Maalouf, N., Nissan, A., Sandbank, J., Yehuda-Shnaidman, E., Ponath, F., Vogel, J., et al. (2020) Breast cancer colonization by Fusobacterium nucleatum accelerates tumor growth and metastatic progression. *Nat. Commun.* 11, 3259

118. Chen, Y., Peng, Y., Yu, J., Chen, T., Wu, Y., Shi, L., Li, Q., Wu, J., and Fu, X. (2017) Invasive Fusobacterium nucleatum activates beta-catenin signaling in colorectal cancer via a TLR4/P-PAK1 cascade. *OncoTarget* 8, 31802–31814

119. Arif, M., Wei, J., Zhang, Q., Liu, F., Basurto-Islas, G., Grundke-Iqbal, I., and Iqbal, K. (2014) Cytoplasmic retention of protein phosphatase 2A inhibitor 2 (I2PP2A) induces alzheimer-like abnormal hyper-phosphorylation of Tau. *J. Biol. Chem.* 289, 27677–27691

120. Sontag, J.-M., and Sontag, E. (2014) Protein phosphatase 2A dysfunction in Alzheimer’s disease. *Front. Mol. Neurosci.* 7, 1–10

121. Liu, F., Grundke-Iqbal, I., Iqbal, K., and Gong, C. X. (2005) Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation. *Eur. J. Neurosci.* 22, 1942–1950

122. Tanimukai, H., Grundke-Iqbal, I., and Iqbal, K. (2005) Up-regulation of inhibitors of protein phosphatase-2A in Alzheimer’s disease. *Am. J. Pathol.* 166, 1761–1771

123. Fissolo, N., Haag, S., De Graaf, K. L., Drews, O., Stevanovic, S., Ram-mensee, H. G., and Weisert, R. (2009) Naturally presented peptides on major histocompatibility complex I and II molecules eluted from central nervous system of multiple sclerosis patients. *Mol. Cell. Proteomics* 8, 2000–2010

124. Lampson, L. A., and Hickey, W. F. (1986) Monoclonal antibody analysis of MHC expression in human brain biopsies: Tissue ranging from “histologically normal” to that showing different levels of glial tumor involve ment. *J. Immunol.* 136, 4054–4062