Posttranslational Protein Modifications
CURRENT IMPLICATIONS FOR CANCER DETECTION, PREVENTION, AND THERAPEUTICS

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Over the last several years major advances in sensitive high throughput technologies have been made in the fields of genomics and proteomics. The hunt for diagnostic and prognostic cancer biomarkers exploits these recent technology platforms. Although the recent developments and use of genomics and proteomics offer much promise in the search for molecular markers of early stage cancers, these methods are inadequate to probe the dynamic nature of signaling processes that cells exhibit during their transformation to become neoplastic. The diverse realm of posttranslational modification (PTM) of proteins encompasses many of the critical signaling events occurring during neoplastic transformation. PTMs offer a plethora of candidates for biomarker detection that complement discoveries using strictly proteomics or genomics platforms. Furthermore the potential to pharmacologically impede tumor growth by administration of an agent that interrupts a specific PTM driving oncogenic progression has been the basis of numerous clinical trials currently underway.

To draw greater attention to the opportunities afforded by innovative research in PTMs, a 2-day workshop was conducted August 2002 in Bethesda, MD. The goals of this meeting were to address several topics where PTMs play roles in cancer progression, consider what technologies can be applied to clinical prevention or detection of cancer, and assess what PTMs could be pursued for development of promising surrogate markers. Since that time some advancement has been made in technological developments to study PTMs, identifying the central roles they play in cancer progression, and determining their amenability as either a cancer biomarker or therapeutic target. A limiting factor in PTM research is that technologies to screen vast numbers of molecules for a particular type of modification are often not available, although recent developments of specific probes and multiplexed platforms may now make broader scale PTM surveying feasible (1–6). For the most part, research in PTM as it relates to biomarker discovery has required the study of discreet modifications on specific proteins of importance to cancer biology. This one by one approach clearly takes time, but the rewards are not to be underestimated in terms of application to cancer detection and treatment.

This review highlights the areas where PTMs are of prime importance for cancer diagnosis and treatment. Table I lists many of the more prominent examples where PTM of specific proteins has relevance toward these aspects of clinical practice in oncology. This review highlights the major aspects of PTMs currently investigated by many laboratories. By providing this overview, it should be apparent that PTMs are key to understanding cancer biology and thus should receive special attention for applications in translational research. PTMs that are currently most germane to clinical applications in cancer medicine are briefly introduced below.

PTMS RELEVANT FOR CLINICAL APPLICATIONS

Phosphorylation—The role of phosphorylation in regulating enzyme activity has long been recognized. Little introduction is needed for this form of PTM as its involvement in intermediary cell metabolism is common knowledge. Defined signaling pathways where aberrant regulation of phosphorylation contributes to oncogenesis include receptor tyrosine kinases/PI 3-kinase/Akt/mTOR, receptor tyrosine kinases/Ras/Raf/MEK/ERK, MEKK/MKK/JNK, and JAK/STAT. All of these signaling cascades, where phosphorylation occurs at nearly each step, have profound control in cell growth, survival, apoptosis, or responses to various extracellular signals. The exploitation of these phosphorylation events for diagnostic and therapeutic intervention in cancer treatment has been a rapidly developing area over the last few years.

Acetylation—The role of acetylation appears analogous to that of phosphorylation. By virtue of neutralizing surface charges on lysine residues, acetylation can regulate protein function or its association with other proteins. In the special case of histones described in depth later, acetylation affects the nature of the association of this abundant class of proteins with DNA.

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1 The abbreviations used are: PTM, posttranslational modification; CFG, Consortium for Functional Glycomics; EGFR, epidermal growth factor receptor; GP73, Golgi protein 73; HDAC, histone deacetylase; ILK, integrin-linked kinase; PDGFR, platelet-derived growth factor receptor; PI, phosphatidylinositol; PIP3, phosphatidylinositol 3,4,5-trisphosphate; mTOR, mammalian target of rapamycin; MAP, mitogen-activated protein; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; ERK, extracellular signal-regulated kinase; MEKK, MEK kinase; MKK, mitogen-activated protein kinase kinase; JNK, c-Jun NH2-terminal kinase; JAK, Janus kinase; STAT, signal transducers and activators of transcription; CDK, cyclin-dependent kinase; E2, ubiquitin carrier protein; E3, ubiquitin-dependent protein isopeptide ligase; PTEN, phosphatase and tensin homolog.

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**Posttranslational Modifications in Cancer**

**Examples of PTMs on proteins contributing to oncogenesis or being used in the context of a cancer biomarker**

All proteins are subdivided by their subcellular localization as indicated to show principal compartments where various PTMs are found to occur.

| Posttranslational modification | Nuclear | Cytosolic, intracellular organelles | Plasma membrane, secreted |
|-------------------------------|---------|------------------------------------|---------------------------|
| Phosphorylation               | pRBs, p53, histones, HDACs, STAT-3 | PTEN, Akt, MAP kinases, death-associated protein kinase, cyclin-dependent kinases | EGFRs, PDGFR, Abl, ILK, osteopontin |
| Glycosylation                 | GP73    |                                    | CD44; galectins; CA125, CA19-9; MUC1, MUC4, MUC16; prostate-specific antigen; osteopontin |
| Ubiquitination, sumoylation   | p53, NF-κB, histones, HDACs | Inhibitor of apoptosis proteins Ras, Rho, Braf | G-protein-coupled receptors |
| Prenylation                   | Histones, DNA polymerase β |                                    |                           |
| Methylation                   | Histones, DNA polymerase β |                                    |                           |
| Acetylation                   | p53, GATA transcription factors, histones, HDACs, NF-κB | | |

**Methylation**—The importance of methylation in protein function and tumorigenesis is still a field in its infancy. Methylated lysine residues still carry a positive charge and thus apparently have little effect on protein conformation. However, lysines that are di- or trimethylated are not accessible for other forms of modification such as acetylation or ubiquitination. It is this distinguishing property that implicates methylation as being important in the regulation of proteins by others forms of PTM.

**Prenylation**—Activation of GTPases such as Ras, Rho, and G-proteins coupled to cell surface receptors is a feature common to many cancers. An obligatory step in their activation is prenylation of a cysteine residue near the carboxyl terminus conferring membrane association of these proteins. This modification entails covalent thiolation with either a 15-carbon farnesyl or a 20-carbon geranylgeranyl isoprenoid group serving as the anchor for membrane attachment. Further processing occurs where the terminal three amino acids are removed by a protease, and the resulting carboxyl-terminal, alkylated cysteine residue is subsequently methylated yielding an isoprenylcysteiny carbamyl methyl ester (7). Following this series of modification reactions, the GTPase protein is anchored to the inner leaflet of the plasma membrane providing the topology necessary for its signal transduction activity.

**Ubiquitination and Sumoylation**—Ubiquitination is a PTM that likely affects all proteins at some point in their life cycle. The most common role ubiquitination plays is in tagging of proteins for degradation via the 26 S proteasome. This signal for degradation usually involves polyubiquitination of a protein. In contrast, monoubiquitination is believed to serve as a regulatory modification of the protein in much the same way phosphorylation regulates protein activity (8). SUMO1 is a ubiquitin-like protein that likewise can be covalently attached to target proteins presumably serving a modulatory function (9). The roles ubiquitination and sumoylation play in tumorigenesis are still poorly understood and perhaps underappreciated; however, cases of ubiquitin ligases showing relationships with oncogenesis are now being uncovered (10, 11).

**Glycosylation**—Nearly all cell surface and secreted proteins are glycosylated. Proper conformational folding of the translated polypeptide chain is facilitated by glycosylation events, and thus protein function is often dependent on or refined by the carbohydrate moieties attached to the polypeptide. Heterogeneity often exists in the multiple oligosaccharide chains attached to a single protein. The structures of these oligosaccharides are dictated by the panel of highly specific glycosyltransferases and glycosidases present in the endoplasmic reticulum and Golgi apparatus of the cell. Alterations in the expression of these enzymes will result in changes of the glycomic profile found on its glycoproteins. Because neoplastic cells show altered transcriptomic profiles, often resembling more embryonic cellular states, the glycome synthetic machinery of the cell is a module often changed by oncogenic transformation. It is perhaps no wonder that many tumor-specific antigens have been discovered to be cell surface carbohydrate structures (12).

**Other Forms of PTM**—There are many other forms of PTM that have been identified; however, their application for cancer medicine is not yet readily apparent. It would be worthwhile to list these PTMs as in the near future they may prove valuable in our understanding of cancer biology and how this disease might be combated. These other modifications include disulfide bond formation, myristoylation, proline isomerization, ADP-ribosylation, transglutamination, citrullination, sulfation, and glycosylphosphatidylinositol anchoring. Moreover, a distinct but common type of glycosylation on cytoplasmic and nuclear proteins has been identified where N-acetylgalactosamine is linked to serine or threonine residues (13).

**PTM and CARCINOGENESIS**

Alterations in gene expression, activation of certain cellular signaling pathways, enhanced proliferation, and dysregulation of cell division or death have long been recognized as hallmarks of cancer progression. PTMs play pivotal roles in all of...
these activities because it is the chemical modifications of key regulatory or structural proteins that dictate the activation state for most cell physiological events. This section describes a number of well established examples where carcinogenesis is dependent upon select proteins subjected to, or participating in, PTM resulting in the aberrations of cell physiology, structural integrity of cellular components, and control of gene expression.

PTM of Nuclear Proteins

Because the nucleus is the site where genetic information is unfolded to enact transcriptomic programs, PTM of nuclear proteins poses a tangible link with tumorigenesis. Two prominent examples that portray how various PTM mechanisms play central roles in tumor biology are the p16/pRB/cyclin D1 and p19/p53/MDM2 cell cycle control pathways. At least one of these pathways appears to be inactivated in all tumors, and the ability to probe into these specific PTMs is shedding light on early stages of carcinogenesis.

pRB—The retinoblastoma gene RB-1 is among the first tumor suppressors to be discovered. The protein product of this gene (pRB1) prevents progression of a cell into S phase by binding to transcription factors of the E2F family and repressing genes involved in nucleotide and DNA synthesis (14, 15). This repressor activity is relieved when cyclin D1 binds cyclin-dependent kinase CDK4 to then phosphorylate pRB1 causing it to dissociate from E2F permitting this transcription factor to be an activator for genes of DNA synthesis. The cyclin-dependent kinase inhibitor p16 is another tumor suppressor of this control pathway that acts to inhibit the activity of the cyclin D1-CDK4 heterodimer. Phosphorylation of pRB1 is thus the key regulatory step in this pathway controlling cell division. Amplification of the cyclin D1 gene or mutations in p16 are often found in tumors contributing to a hyperphosphorylated state of pRB1 and a consequential commitment to continued cell growth (16, 17).

Chromatin Structural Alterations in Carcinogenesis

At least two other proteins have been identified in the pRB family, all showing similar mechanisms of modulation by phosphorylation and cyclins in regulating E2F factors (18). Reduction of pRB2 expression by promoter methylation has been reported recently to contribute to retinoblastoma tumors and non-small cell lung cancer (19). These findings draw clear parallels with the more widely known roles of aberrant pRB1 activity in many cancers.

p53—In a manner similar to that seen with pRB1, p53, another tumor suppressor, is subject to multiple modes of PTM. Normally p53 is maintained at low levels due to ubiquitination by the ubiquitin E3 ligase MDM2 and ensuing degradation; however, upon exposure to certain stressful stimuli, p53 ubiquitination is suppressed leading to formation of an active tetrameric p53 complex triggering regulation of a host of genes controlling DNA repair, apoptosis, and cell cycle arrest (20). In tumor cells MDM2 is often amplified thus constitutively supporting p53 degradation preventing normal cellular responses to stress, whereas inhibition of MDM2-p53 association reverses this effect (21). Recently specific mutations of p53 have been found to foster its hyperubiquitination (22) revealing a likely mechanism by which p53 mutations contribute to neoplasia. Other forms of PTM that regulate p53 levels and activity are acetylation and phosphorylation. Acetylation appears to prevent ubiquitination, whereas p53 deacetylation promotes its ubiquitination and subsequent degradation. The existence of at least 17 phosphorylation sites contributes to how p53 controls transcriptional activity.

Other Transcription Factors and Nuclear Proteins—The importance of PTMs in gene regulation and carcinogenesis can easily be extended to the vastly diverse set of other nuclear proteins. For example, histone acetyltransferases can act upon numerous transcription factors such as p53, members of the GATA family, many nuclear receptor superfamilies, and a host of co-activators (23). It should not be surprising that many of the enzymes that catalyze PTMs on other proteins are themselves subject to different forms of PTM. For example, histone deacetylases are subject to regulation by phosphorylation and sumoylation (24). In essence, nearly every nuclear protein is fair game to be modified in some way as a means of modulating its activity. Inherent in this is the diversity of PTMs that remain to be characterized on such a wide ranging population of host proteins and understanding the consequences they dictate for nuclear function and how this leads to oncogenesis.
The state of histone acetylation has broad influence on chromatin structure, nucleosome packing, and hence the transcriptional states of specific genes. Two groups of enzymes responsible for regulating the reversible and dynamic state of histone acetylation are histone acetyltransferases and histone deacetylases (HDACs). Because histones contain a high degree of basic amino acids, acetylation serves to neutralize the abundance of positive charges and decrease histone affinity for the phosphate backbone of DNA. Acetylation of histones, in general, leads to transcriptional activation as nucleosomes unpack from the 30-nm chromatin fibers (Fig. 1), and other transcriptional regulatory proteins now gain access to promoter elements on DNA. As most tumors are characterized by nuclei with a higher degree of euchromatin, normally indicative of unpacked chromatin structure, a significant factor contributing to this phenomenon is histone acetylation.

The development of a battery of HDAC inhibitors, discussed in greater detail later in this review, highlight the importance of histone acetylation in cancer progression.

Methylation is another PTM commonly found on histones having significant roles in chromatin remodeling. Methylation may be a mechanism to prevent acetylation on lysine residues. Histone methyltransferases utilize S-adenosylmethionine as a methyl donor; however, its unmethylated analogue S-adenosylhomocysteine can decrease histone methyltransferase activity. The role diet can play in this process is of interest as the composition of dietary intake, namely folic acid content, can influence the ratio of S-adenosylmethionine:S-adenosylhomocysteine. The link between diet and cancer is widely recognized, and the role of folic acid as a methylation cofactor certainly has broad implications not only for the activity of histone methyltransferases but also other biochemical processes that are dependent on methyl group donors such as DNA mutations arising from misincorporation of dUTP into DNA and methylation of DNA resulting in gene silencing (25).

Several of the major PTMs histones can undergo have been discussed, but the list is longer than discussed here. Phosphorylation, ubiquitination, and sumoylation have also been demonstrated. Recently histone lysine demethylases have been discovered posing yet another level of modulation of chromatin remodeling (26). Because there are multiple sites on the histone tails that can accommodate various forms of PTM, some effort has been devoted to understanding how modifications on defined amino acids or influenced in the context of other modifications concurrently existent on the histones.

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**Modulation of Signal Transduction Pathways**

Altered regulation of signal transduction pathways often plays principal roles in the growth properties of neoplastic cells. Targeted intervention to correct aberrant signaling is a plausible means to reverse the effects of altered signaling mechanisms in cancer cells. Signaling cascades often involve protein phosphorylation of key intermediary regulatory kinases and metabolic enzymes. Markers to identify aberrant signaling are rapidly expanding in light of their clear relationship to growth of neoplastic cells. The paragraphs that follow present some hallmark cases typifying where protein phosphorylation plays some well established roles in neoplastic growth.

**PTEN**—Deregulation of the pathways using phosphatidyli-
in ositol 3,4,5-trisphosphate (PIP₃) as a second messenger is common to many types of tumors. The tumor suppressor PTEN is a phophatase that dephosphorylates PIP₃ to phosphatidylinositol 4,5-bisphosphate serving to keep signaling by this second messenger in check. The activity and stability of PTEN is dependent upon phosphorylation near its carboxyl terminus (28). In its dephosphorylated state, PTEN is activated to enzymatically degrade PIP₃, but PTEN is also quite unstable in the dephosphorylated state, apparently subject to proteasomal degradation. When it is phosphorylated, PTEN recruits binding of another tumor suppressor, PICT-1, conferring greater stability and protection from degradation (29). Approximately 20% of tumor-associated PTEN mutations disrupt the phosphorylation sites at the carboxyl-terminal region resulting in rapid degradation of this critical enzyme.

Receptor Tyrosine Kinases—Because many protein kinases participate in signal transduction cascades, great attention has been drawn to tyrosine kinase inhibitors in relation to therapeutic treatment of cancer. The epidermal growth factor receptor (EGFR) subfamily, platelet-derived growth factor receptor (PDGFR), and c-Kit receptor among others all belong to the tyrosine kinase superfamily. Upon dimerization these receptor (PDGFR), and c-Kit receptor all belong to the EGFR subfamily, platelet-derived growth factor receptor (PDGFR), and c-Kit receptor among others all belong to the tyrosine kinase superfamily. Upon dimerization these receptors autophosphorylate to promote binding and tyrosine phosphorylation of other intracellular signaling proteins such as Src, phospholipase C and Kinesins, leading to enzymatic degradation of other signaling proteins such as Src, phospholipase C and Kinesins, leading to enzymatic degradation of their protein kinase B/Akt substrates. Downstream signaling events can then occur in a multitude of pathways that regulate cell growth or apoptosis. These pathways include the participation of such protein kinases as mitogen-activated protein (MAP) kinases and protein kinase B/Akt.

Cytokine receptors represent another group of tyrosine kinases known as the Janus kinases or JAKs often implicated in cancer. Following autophosphorylation these kinases bind and phosphorylate a group of latent regulatory proteins termed STATs, which translocate to the nucleus to activate transcription of certain genes that can influence cell proliferation and survival.

Integrin-linked Kinase—Integrin-linked kinase (ILK) has signaling properties that tie integrins and growth factors to downstream pathways such as protein kinase B/Akt phosphorylation; activation of β-catenin, cyclin D1, and AP-1 pathways; and expression of matrix metalloproteinase-9, which functions to degrade extracellular matrix to promote the invasion by cancer cells (30, 31). ILK expression is elevated in several cancers so targeted inhibition of this enzyme may provide a mechanism to treat cancer. ILK inhibitors have been reported to increase survival in a rat orthotopic model of pancreatic cancer (32). Because of the host of interactions ILK poses in regulating cell adhesion and extracellular matrix interactions with different signaling pathways, the potential of this protein kinase as a biomarker and therapeutic target should be investigated further.

Ras—Activation of MAP kinase pathways in tumors stimulates cellular proliferation. Upstream regulators commonly found to be a culprit in this aspect of oncogenic transformation are members of the Ras family (33). Activation of Ras is dependent on prenylation, thus making this key enzymatic reaction a target for antineoplastic drugs for therapeutic use in a wide range of tumors. Subsequent sections in this review will elaborate further on how Ras prenylation is under investigation both diagnostically and therapeutically.

Molecular Alterations in Cell Surface Architecture

Altered carbohydrate profiles on the cell surface are a property common to apparently all tumors. The outer glyocalyx found on epithelial and mesenchymal cells serves multiple roles over a broad scope of cell interactions with its microenvironment such as hysteresis protection, external molecular buffering, adhesion to extracellular matrix, and intercellular adhesion. The latter two functions in particular are paramount in metastasis and invasion. Pronounced alterations in glycan profiles clearly contribute to the ability of a cell to detach from its normal tissue site and possibly adhere within another organ site. For example, sialyl Lewis structures, which are often found in tumors, show a propensity to bind selectins likely conferring the metastatic properties of these cells (34). Thus, the implications of carbohydrate interactions at the cell surface are profound as they seem to dictate many aspects of metastatic and invasive cell behavior.

PTMS AS CANCER BIOMARKERS

Phosphoproteomics—Aberrant activation of defined signal transduction pathways typically bestows altered growth properties to tumors. Understanding which pathways are activated might enable a clinician to make rational decisions concerning prognosis and devise a strategy for treatment. The phosphoproteomic profile of a tumor provides an important biomarker panel pertinent to this tactic. With the advent of a vast repertoire of antibodies specific to defined phosphorylated sites of proteins participating in signaling pathways, innovative protein microarray platforms are being developed to characterize tumors by quantifying the phosphorylation status of many signaling proteins (5). This type of analysis allows one to determine the activation status of multiple signaling pathways providing valuable insights into establishing an individualized targeted therapy for the patient (35, 36). This analysis, in principal, could be continued following the initial stages of treatment to monitor whether the therapy is effective and provide follow-up strategies to modify treatment for recurrent tumors.

An interesting set of studies by two independent groups highlights how determination of the phosphorylation status of protein kinase B/Akt can be an important biomarker in predicting the response of gliomas to tyrosine kinase inhibitors (Fig. 2). One group discovered that EGFR expression and amplification characterized responders to erlotinib, whereas phosphorylation of protein kinase B/Akt precluded efficacy of the drug (37). This finding was apparently corroborated by a
EGFR, symbolized by the.

Erlotinib inhibits tyrosine kinase activity of whereas tumors exhibiting phosphorylated protein kinase B (PKB/Akt) show EGFR expression or amplification and PTEN expression, blastomas. Glioblastomas responsive to tyrosine kinase inhibitors.

Second group where coexpression of EGFR and PTEN in tumors of glioblastoma patients was associated with clinical efficacy to tyrosine kinase inhibitors, whereas EGFR-positive tumors that did not express PTEN were not responsive to drug therapy (38). It is hypothesized that PIP3 production leads to protein kinase B/Akt phosphorylation thus driving glioma growth. PIP3 production is stimulated by EGFR activation; however, PTEN acts to keep this second messenger in check. Therefore, EGFR inhibition by erlotinib is only effective when PTEN is expressed to prevent these downstream signaling effects on cell growth.

**Prenylation**—Detection of farnesylated proteins as potential cancer biomarkers has not been widely explored. Numerous cancers, however, are characterized by mutations in Ras proteins leading to persistent activation of their affected pathways. Identification of tumors bearing mutations in these GT-Pases can thus serve as a prognostic marker to predict which cancers may respond to chemotherapeutic agents interfering with prenylation.

**Glycomics**—Complex carbohydrates offer a fertile class of molecular structures to serve as biomarkers of oncogenesis. Nevertheless in the hunt for biomarkers, proteomics and genomics technologies have been at the forefront primarily due to facile methods to analyze or manipulate these biopolymers. Although proteins and nucleic acids, in essence, represent a one-dimensional polymeric code, the structure of complex carbohydrates by comparison is multidimensional. Multiple linkage sites and two different configurations of each linkage can result on each saccharide unit along with the possibility of having branch points on select units. Due to this more intricate chemostructural language requiring sophisticated analytical chemical methods, the field of glycomics has received less attention than that of proteomics and genomics with regard to biomarker discovery.

The changes in glycosylation of glycoproteins occurring as a result of oncogenic transformation can involve over- or underglycosylation or expression of "novel" glycan structures. These changes result from the altered expression of glycosyltransferases by the transformed cells where overglycosylation and “neoeexpression” are normally due to elevated expression or induction of specific glycosyltransferases. Underglycosylation is often due to repression of specific glycosyltransferases. Structures of glycans commonly found on cancer cells are depicted in Fig. 3.

The mucin family has been a prominent player in the cancer biomarker field. These glycoproteins are either secreted or integral cell membrane proteins at the apical surface of epithelial cells. Their amino acid sequences have several tandem repeats that serve as sites for O-glycosylation. In addition, they also bear many N-linked oligosaccharides. The carbohydrate content of the secreted mucins represents about 25% of their total mass conferring them with hydrodynamic properties believed to impart lubrication and protection at the luminal cell surface. In several types of cancer, overexpression of mucins has been noted; these mucins typically display abnormal or incomplete glycosylation (39, 40). The ovarian carcinoma biomarker CA125 is a mucin where only relatively recently has the repertoire of its diverse oligosaccharide structures been elucidated (41). Despite the use of an ovarian cancer cell line as the source for CA125, the glycomic profile of this densely glycosylated protein was found to be very heterogeneous with nearly 20 N-glycans and about another 20 O-glycans identified. It remains to be determined which, if any, of these glycans represent cancer-specific oligosaccharides as the glycan structures from CA125 of normal tissue remain to be reported. The rigorous effort that this study used and the complex glycomic profile encountered exemplify the difficulties in studying the full scope of protein glycosylation. Undoubtedly a wealth of cancer biomarker candidates is likely to be represented by the variations in glycoforms representing glycoprotein species produced by neoplastic cells.

In a recent study searching for serum biomarkers of hepatocellular carcinoma, a novel glycomics profiling approach was taken by isolating the total population of N-linked oligosaccharides from serum glycoproteins, comparing chromatographic glycan profiles of control versus cancer subjects and determining structures of any oligosaccharides unique to cancer-derived samples (42). Upon identification of a specific oligosaccharide showing an atypical α-1,6-linked fucosyl on the core structure, the glycoproteins bearing this modification were then purified and identified. Golgi protein 73 (GP73) was a major constituent showing this particular glycan. Interest-
ingly GP73 is not normally a secreted protein, thus its presence in blood (43) in combination with an atypical glycan structure, possibly reflects a pathological condition of the neoplastic cells. Moreover additional hyperfucosylated proteins in serum from liver cancer patients were identified among which the current hepatocellular carcinoma biomarker H9251-fetoprotein was found. This study could pioneer a new approach for disease diagnosis and biomarker discovery as our technology to analyze glycomic profiles develops.

Activity-based Profiling—In concert with targeted therapy for enzymes producing PTMs, another approach that can be taken is called activity-based protein profiling. This technology seeks to directly measure the activity of a particular enzyme or class of enzymes. Its utility to study PTMs is 2-fold. First, an enzyme that produces a particular PTM may be monitored directly by its activity. Second, because an enzyme is often regulated by PTM, measurement of its activity can be an indirect marker correlated with its PTM status.

To successfully perform activity profiling, the active site of the enzyme is targeted to bind specifically designed chemical probes that then beacon its catalytic function. Proteases, kinases, and phosphatases have been investigated by this approach. Serine hydrolases, a class of protease, and sialyl acetylersterase, an enzyme modifying sugar moieties of glycoproteins, were analyzed by certain fluorophosphonate probes to characterize breast and melanoma cancer cell lines (44, 45). Distinctions in their subcellular distribution and activity expression patterns within each class of enzymes were found to distinguish the source of tumors and predict the invasive nature of the cancer cells. More recently a diversity of metalloproteases have been studied by this approach by developing a library of probes for this enzyme class (46) to gain insight into the invasive properties of the cells.

Another paradigm to measure enzyme activity associated with PTMs is to devise assays that directly measure these activities. A variety of kinase profiling kits, microarrays, or screening services are now commercially available to rapidly identify and quantitate the activities of specific protein kinases. An innovative microarray platform utilizing electrochemiluminescence to measure ubiquitin ligase activity has recently been developed to study E2 and E3 ubiquitin ligases (4). Throughout all these examples the advantages of activity-based profiling become apparent as a physiologic property of the tumor is directly measured rather than simply detecting the presence of a particular molecular marker.

**PTMs IN CANCER TREATMENT**

The direct roles that PTM events play in cancer progression makes targeted therapy a feasible option in cancer treatment. If select pathways requiring PTM or the involvement/removal of key proteins subjected to PTM serve to promote oncogenic growth, then perturbation of these key PTM events could prove effective in slowing its growth or facilitating cell death. This section highlights several examples where PTMs are being exploited for therapeutic intervention. Table II lists drugs that perturb specific PTM reactions currently under clinical trials or approved by the United States Food and Drug Administration.

**HDAC Inhibitors**—As discussed earlier, the state of histone acetylation seems to be a major factor in chromatin remodeling and regulation of gene expression. HDACs serve to deacetylate histones and thereby promote nucleosomal re-packing and transcriptional repression. Although one might surmise in a broad sense that HDAC activity should inhibit

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**Figure 3.** Glycoprotein glycan structures associated with cancers. The terminal Lewis structures shown are often highly expressed in cancer on N- or O-linked glycans as well as on glycolipids. FcaA2G2 is not specific to cancer but has been found as a unique glycoform associated with several proteins in hepatocellular carcinoma (42) where the normal glycoform does not contain the fucose residue. The β1–6 branching glycans are highly expressed in cancers where N-acetylglucosaminyltransferase V is up-regulated. Tn and sialyl-Tn are aberrant glycans found on mucins as a result of underglycosylation. Gal, galactose; GalNac, N-acetylgalactosamine; Man, mannose; GlcNAc, N-acetylgalactosamine; Fuc, fucose; NeuAc, neuraminic acid (sialic acid)
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TABLE II

| Drug                                      | Protein target                                      | Current status: clinical indication                  |
|-------------------------------------------|-----------------------------------------------------|-----------------------------------------------------|
| Protein kinase inhibitors                  |                                                     |                                                     |
| Erlotinib (Tarceva)                       | EGFR                                                | FDA-approved (Tarceva): non-small cell lung cancer   |
| Gefitinib (Iressa)                        | EGFR                                                | Phase II: glioblastoma, breast cancer                |
| Imatinib (Gleevec)                        | PDGFR/Ab1/Kit                                       | FDA-approved: chronic myeloid cancer                 |
| UCN-01-7 (7-hydroxystaurosporine)         | Cyclin-dependent kinases                            | Phase I: multiple tumor types                       |
| BAY 43-9006                               | Raf kinase                                           | Phase I: multiple solid tumors                      |
| E7070                                     | CDK2, Cyclin E                                      | Phase II: squamous cell carcinoma                   |
| SU5416                                    | Vascular endothelial growth factor receptor, c-Kit, FLT3 | Phase II: acute myeloid leukemia                    |
| Histone deacetylase inhibitors            | Histones, transcription factors (likely)            | Phases I and II: multiple tumors                    |
| MS-275, CI-994, Pivanex, valproic acid, depsipeptide, suberoylanilide hydroxamic acid |                                                     |                                                     |
| Prenylation inhibitors                    |                                                     |                                                     |
| Tipifarnib (Zarnestra), lonafarnib (Sarasar) | Farnesyltransferase (Ras prenylation)               | Phases II and III: multiple myeloma, multiple tumor types |
| Ubiquitination-proteasome inhibitors      | 26 S proteasome                                      | FDA-approved: multiple myeloma                       |
| Bortezomib (Velcade)                      |                                                     |                                                     |

Table: Examples of drugs that selectively inhibit PTMs currently under clinical trials or that have passed United States Food and Drug Administration (FDA) approval are shown. Antibodies to receptor tyrosine kinases have not been included in this table.

neoplastic growth, this assumption is too simplistic as a more refined control of histone acetylation and deacetylation takes part in controlled cell growth (47). This point is exemplified by a number of HDAC inhibitors currently in Phase I or II clinical trials that illustrate the impact of these PTM enzymes on cancer progression (48). These inhibitors display growth arrest, promote differentiation, or induce apoptosis by altering expression of select genes. Specific examples reflecting these effects of altered gene expression by HDAC inhibitors are increased transcription of p21 and thioredoxin-binding protein-2 (49, 50). The former is a cyclin-CDK inhibitor promoting arrest of cells in G1, whereas elevated thioredoxin-binding protein-2 results in reduced levels of thioredoxin, a cofactor necessary for transcription. It would appear by these examples that the therapeutic effect of HDAC inhibitors in stunting tumor growth is by supporting transcription of several tumor suppressor genes that would otherwise be repressed via chromatin remodeling in the transformed cells. Unfortunately HDAC inhibitors are also rather toxic probably due to the fact that little control can be applied to selecting which nucleosomes should be targeted. A strategy to target PTM of regulatory proteins of the nucleus controlling expression of key gene networks might be a more reasonable approach to take.

**Tyrosine Kinase Inhibitors**—Receptor tyrosine kinases are often found guilty in contributing to the proliferative nature of oncogenic cells. Antibodies directed to the extracellular domains of these receptors have demonstrated efficacy in cancer therapy (51). A number of small molecule inhibitors that target the ATP binding pocket of the tyrosine kinase domain have also been designed and tested. EGFR inhibitors gefitinib (Iressa) and its congener erlotinib (Tarceva) have been used extensively in clinical trials for treatment of non-small cell lung cancer (52), but other tumors dependent on EGFR have also been tested including breast cancer (53) and gliomas (37, 38). For non-small cell lung cancers, gefitinib did not improve patient survival (54); however, the results with erlotinib have shown more promise (55). In similar fashion, the PDGFR/Ab1/Kit inhibitor imatinib (Gleevec) has shown positive response in clinical trials on the treatment of myeloid leukemia and gastrointestinal stromal tumors (56). The mechanism of action of these agents is via inhibition of receptor autophosphorylation suggesting that targeted therapy may show promise in the treatment of cancers dependent on these dysregulated signaling cascade systems.

Further investigation into the actions of gefitinib and erlotinib for the treatment of non-small cell lung cancers has revealed some interesting molecular features contributing to the etiology of lung adenocarcinomas. A series of distinct somatic mutations in the tyrosine kinase domain of EGFR were discovered arising in patients who were non-smokers, showing higher prevalence in women of oriental background (57–59). Often these mutations are accompanied by amplification in the EGFR gene. Interestingly several cases have been reported where the effectiveness of these EGFR inhibitors had ceased in some patients who were previously responsive to the drugs. The recurrent tumors from these patients were subsequently found to have developed a second mutation in the tyrosine kinase domain rendering the drug binding pocket unaccommodating to the inhibitor (60, 61). These compelling findings suggest that responsiveness of lung adenocarcinomas to these therapeutic agents may be predicted by the sequence of the EGFR tyrosine kinase domain. This type of reasoning is likely applicable to other can-
cancers exhibiting mutations in common. To better address these possibilities the National Cancer Institute (NCI) and National Human Genome Research Institute are cofunding a pilot project to map mutations of the human cancer genome called The Cancer Genome Atlas (cancergenome.nih.gov). The mission of this project is to comprehensively identify the underlying genomic roots that repeatedly arise for different forms of cancer from which more effective therapeutic strategies might be devised.

Farnesyltransferase Inhibitors and Statins—Because mutant Ras proteins are dependent on prenylation for their activation in promoting oncogenesis, considerable effort has been invested designing inhibitors of various stages of this process. A number of farnesyl protein transferase inhibitors have already entered Phase II and III clinical trials for tumors driven by Ras (62). More recently, drugs targeted for the subsequent proteolytic cleavage and carboxyl methylation enzymes involved in the latter steps of prenylation are being investigated; however, only minimal effects on tumor growth have been observed (7), suggesting prenylation alone is primarily sufficient for GTPase activation. Another group of studies currently in early stages is exploring inhibitors designed for the prenyl-binding domains of proteins that interact with these GTPases to mediate their signal transducing activities (63).

Related to inhibition of prenylation is the finding that a modest secondary benefit of cholesterol-lowering statins has been observed for a number of cancers driven by Ras expression (64). The chemotherapeutic rationale behind the benefit of statins is that because these drugs inhibit synthesis of isoprenoid precursors, a reduction of not just cholesterol but also farnesyl renders the tumorigenic cells susceptible to apoptosis due to diminished prenylation of Ras protein.

Ubiquitination and Proteasome Inhibition—The success of the drug bortezomib in clinical trials for treatment of multiple myeloma highlights the importance of ubiquitination in cancer biology (65). Bortezomib is a 26 S proteasome inhibitor promoting cell cycle arrest and apoptosis presumably by preventing degradation of critical transcription factors, tumor suppressors, and other regulatory proteins that may be subject to polyubiquitination during oncogenic progression. As better tools are developed to study ubiquitination, we should expect to find candidates for cancer biomarkers and leads to additional drug targets based on this type of PTM.

Vaccines Based on Carbohydrate Epitopes—Presentation of aberrant complex carbohydrates on the cell surface of tumor cells opens the possibility to trigger the immune helper T cell response to eliminate cells bearing such structures. This paradigm has prompted many studies seeking to develop vaccines exploiting characteristic tumor-associated glycan structures such as Lewis y, sialyl-Tn, Globo H, and carbohydrates found on glycolipids (66–69). Several of these immunotherapeutic agents have reached Phase II and III clinical trials. New innovations are continuously seeking to adapt novel carrier scaffolds on which to chemically link carbohydrate antigens to minimize carbohydrate epitope suppression and maximize cytolytic immune response directed to the glycan structure.

Combination Therapies—The ability to attack tumors at more than one critical facet of their biology often improves the effectiveness of treatment. With a wide range of investigational drugs that exploit essential PTM events commonly required by many tumors, combination therapy is being used with various other forms of treatment. These include the use of various PTM inhibitors with chemotherapeutic adjuvants such as taxanes, nucleoside analogs, and platinum agents (70–72); radiation therapy (73); combination therapy with estrogen receptor antagonists (53); and finally combinations of drugs that inhibit different forms of PTM (74, 75). In several cases prolongation of patient survival was found when these combination therapies were used. With these promising results it appears that the addition of PTM inhibitors to our chemotherapeutic arsenal will prove valuable in the battle against cancer.

CONSORTIUM-BASED INITIATIVES AND APPROACHES FOR PTMS

Several funding initiatives to promote technology development to study PTMs have recently been introduced by the National Institutes of Health. The Clinical Proteomics Technologies for Cancer initiative funded by NCI seeks to foster an infrastructure to establish standardized reagents, specimens, and protocols for the high throughput analysis of the proteome. Included in this mission is the necessity to detect and fully characterize PTMs that exist within the proteome. Since the inception of this funding initiative will occur near the time this review is published, the success of this program in advancing our ability to analyze PTMs will not be determined for several years.

The National Center for Research Resources has funded a Proteomic Research Resource for Integrative Biology that complements the NCI effort in clinical proteomics. Technical capabilities for characterization of complex proteomic systems at this center include liquid chromatography technology coupled to mass spectrometry instrumentation to enable identification of PTMs in complex peptide mixtures. This site also develops single chain antibodies for characterization of PTMs.

The field of glycomics has received considerable attention from several National Institutes of Health institutes to bolster technologies to analyze complex carbohydrate structures. The National Institute of General Medical Sciences has funded the Consortium for Functional Glycomics (CFG), consisting of over 200 investigators internationally. The CFG cores have developed new technologies and public databases for high throughput structural analysis of the human and mouse glycomes, glycogene microarray screening, and glycan array analysis for high throughput carbohydrate binding analysis. The unique infrastructure developed by the CFG lends itself well to the immediate support of a number of
glycomics approaches for the discovery of glycan-based cancer biomarkers. In addition, the National Center for Research Resources is now supporting four Glycomics and Glycotechnology Resource Centers with the primary mission to develop effective technologies and methods to analyze glycans and glycoconjugates. Two of these centers are seeking to specifically integrate glycomics technologies with proteomics to analyze glycoproteins in a more robust manner. The NCI is now seeking to leverage these unique resources by offering a new funding initiative to stimulate discovery and validation of glycan-based cancer biomarkers. The Alliance of Glycobiologists for Detection of Cancer and Cancer Risk will be formed through this initiative to fund several tumor glycome laboratories who can partner with the CFG and Glycomics Resource Centers in their biomarker discovery efforts.

CURRENT LIMITATIONS AND FUTURE RESEARCH DIRECTIONS

The developments that are being brought forward in PTM research are promising, but it seems greater emphasis is still needed to more fully exploit this exciting field. Several recommendations were made at the 2002 PTM workshop held in Bethesda, MD to serve as a guide on how advances in PTM research are proceeding in relation to oncological application. At this stage, several years later, it would be appropriate to reflect and consider which if any of these recommendations from the NCI Workshop have been followed. As these topics are considered it should be evident that fulfillment of many of these recommendations are in progress, but clearly this effort would benefit from greater resources devoted to technologies to analyze PTMs.

The first consideration was whether a “forward” or “backward” approach should be taken to associate specific PTMs with cancer. In the forward approach a comprehensive analysis of PTMs from early stage lesions would be performed to determine which events are predictive of early cancer. The backward approach involves beginning with an analysis of cancers and backtracking PTMs to precancerous lesions. Before initiating these types of comprehensive analyses prioritization should be made on specific genes or pathways involving PTMs. The approach taken will depend on the nature of the study. In perusing publications over the last few years it appears that the backward approach has been applied most often as it is difficult to identify which early PTM events in early lesions will lead to onset of cancer. For the most part, our tools to study these early events are probably not robust enough to achieve the sensitivity necessary to identify such subtle changes among the vast host of PTMs that are present within any cell.

To more specifically address how progress can be achieved in our understanding of the relationships of PTMs with cancer progression two main areas were revealed.

1. Technological advancements in high throughput quantitative assays for PTMs are needed. Current technologies need to be used until newer technologies are developed and found to be more effective. Creation of “tool sets” for different classes of PTMs would aid researchers in this research. Focus should be placed on investigating subcellular or protein compartments, macromolecular complexes, and protein-protein interactions. Priority needs to be placed on developing ligands and antibodies to probe PTMs. Tissue resources and animal models for human cancer need to be assessed for applicability to this field of research.

2. More intensive basic research is still needed to gain a better understanding of the roles PTMs play in carcinogenesis; however, progress is continually being made in this arena. The discovery phase of PTMs should be broadly inclusive and encompass a wide array of normal, precancerous, and cancerous specimens including sets of samples from the same individual and from different individuals presenting with the same type of lesion. Large prospective cohort trials are needed in populations having low, average, or high risk of specific cancers.

A final recommendation was given concerning programmatic initiatives to foster PTM research. Targeted funding for basic PTM research, as well as developmental resources, will provide recognition of the importance of proteomics research to cancer and the need to have important tools such as antibodies and ligands. Specialized centers should be promoted to serve as sites where technology specialists work closely with clinical investigators, biological specimen or animal model resources, and informatics resources to advance progress in this field.

These necessities are being met in part by the initiatives described in the preceding section; however, because the proteomics initiatives do not focus solely on PTMs it remains to be determined how effectively these developing technologies will probe the diversity of PTMs and their clinical implications in oncology. Nevertheless it is somewhat reassuring to see that academic laboratories and the private sector have contributed significantly to developing high throughput platforms and antibody probes to study the complex realm of PTMs.

REFERENCES

1. Blixt, O., Head, S., Mondala, T., Scanlan, C., Huflejt, M. E., Alvarez, R., Bryan, M. C., Fazio, F., Calarese, D., Stevens, J., Razi, N., Stevens, D. J., Skehel, J. J., van Die, I., Burton, D. R., Wilson, I. A., Cummings, R., Bovin, N., Wong, C. H., and Paulson, J. C. (2004) Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. Proc. Natl. Acad.
22. Shimizu, H., Saliba, D., Wallace, M., Finlan, L., Langridge-Smith, P. R., and Sengupta, N., and Seto, E. (2004) Regulation of histone deacetylase activ-

24. Nejat, N., and Downes, C. P. (2004) PTEN function: how normal cells control it and tumour cells lose it. Biochem. J. 382, 1–11

26. Okahara, F., Iwakura, H., Kaneko, Y., and Maela, T. (2004) Regulation of PTEN phosphorylation and stability by a tumor suppressor candidate protein. J. Biol. Chem. 279, 45300–45303

28. Persad, S., and Dendar, S. (2003) The role of integrin-linked kinase (ILK) in cancer progression. Cancer Metastasis Rev. 22, 375–384

30. Persad, S., and Dedhar, S. (2003) The role of integrin-linked kinase, a promising cancer therapeutic target: biochemical and biological properties. Pharm. Ther. 93, 233–242

32. Yau, C. Y., Wheeler, J. J., Sutton, K. L., and Hedley, D. W. (2005) Inhibition of integrin-linked kinase by a selective small molecule inhibitor, QLT0254, inhibits the P38/PKB/mTOR, Stat3, and FKHR pathways and tumour growth, and enhances gemcitabine-induced apoptosis in human orthotopic primary pancreatic cancer xenografts. Cancer Res. 65, 1497–1504

34. Fuster, M. M., Brown, J. R., Wang, L., and Esko, J. D. (2003) A disaccharide precursor of sialy Lewis X inhibits metastatic potential of tumor cells. J. Cell. Biochem. 91, 51–77

36. Petricoin, E., Bichsel, V., Calvert, V., Espina, V., Winters, M., Young, L., Belluco, C., Trock, B., Lippman, M., Fishman, D., Sgroi, D., Munson, P., Eberhard, D., Arvold, N. D., Baumber, R., Lamborn, K. R., Kapadia, A., Malec, M., Berger, M. S., and Stokoe, D. (2005) Epidermal growth factor receptor, protein kinase B/Akt, and glioma response to erlotinib. Cancer Res. 65, 1505–1512

38. Mellinghoff, I. K., Wang, M. Y., Vivanco, I., Haas-Kogan, D. A., Zhu, S., Diaz, R., Lee, J. C., Sellers, W. R., Stokoe, D., Prados, M., Cloughesy, T. F., Horvath, S., Liau, L. M., Cavenee, W. K., Rao, P. N., Beroukhim, R., Peck, T. C., Lee, J. C., Sellers, W. R., Stokoe, D., Prados, M., Cloughesy, T. F., Sawyer, C. L., and Mischel, P. S. (2005) Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N. Engl. J. Med. 352, 193–205

40. Moniaux, N., Andrianifahanana, M., Brand, R. E., and Batra, S. K. (2004) Hyperphosphorylation of pRb: a mechanism for RB tumor sup-

42. Leslie, N. R., and Downes, C. P. (2004) PTEN function: how normal cells control it and tumour cells lose it. Biochem. J. 382, 1–11

44. Jessani, N., Humphrey, M., McDonald, W. H., Niessen, S., Masuda, K., Gangadharan, B., Yates, J. R., Ills, M., and Cravatt, B. F. (2004)
Carcinoma and stromal enzyme activity profiles associated with breast tumor growth in vivo. *Proc. Natl. Acad. Sci. U. S. A.* 101, 13756–13761

Jessani, N., Liu, Y., Humphrey, M., and Cravtav, B. F. (2002) Enzyme activity profiles of the secreted and membrane proteome that depict cancer cell invasiveness. *Proc. Natl. Acad. Sci. U. S. A.* 99, 10335–10340

Sieber, S. A., Niessen, S., Hoover, H. S., and Cravtav, B. F. (2006) Proteomic profiling of metalloprotease activities with cocktails of active-site probes. *Nat. Chem. Biol.* 2, 274–281

Benson, L. J., Gu, Y., Yakoveeva, T., Tong, K., Barrows, C., Strick, C. L., Cook, R. G., Muzzen, C. A., and Annunziato, A. T. (2006) Modifications of H3 and H4 during chromatin replication, nucleosome assembly, and histone exchange. *J. Biol. Chem.* 281, 9287–9296

Marks, P. A., Richon, V. M., Miller, T., and Kelly, W. K. (2004) Histone deacetylase inhibitors. *Adv. Cancer Res.* 91, 137–168

Butler, L. M., Zhou, X., Xu, W. S., Scher, H. I., Rifkind, R. A., Marks, P. A., and Richon, V. M. (2002) The histone deacetylase inhibitor SAHA arrests cancer cell growth, up-regulates thioredoxin-binding protein-2, and down-regulates thioredoxin. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11700–11705

Richon, V. M., Sandhoff, T. W., Rifkind, R. A., and Marks, P. A. (2000) Histone deacetylase inhibitors for cancer chemotherapy and hemoprophylaxis. *Exptl. Biol. Med.* (Maywood) 229, 567–585

Rajkumar, S. V., Richardson, P. G., Hideshima, T., and Anderson, K. C. (2005) Proteasome inhibition as a novel therapeutic target in human cancer. *J. Clin. Oncol.* 23, 630–639

Gilewski, T., Ragupathi, G., Bhuta, S., Williams, L. J., Mussell, C., Zhang, X. F., Bornmann, W. G., Spassova, M., Bencsath, K. P., Panageas, K. S., Chin, J., Hudis, C. A., Norton, L., Houghton, A. N., Livingston, P. O., and Danielsen, S. J. (2001) Immunization of metastatic breast cancer patients with a fully synthetic globo H conjugate: a phase I trial. *Proc. Natl. Acad. Sci. U. S. A.* 98, 3270–3275

Krug, L. M., Ragupathi, G., Hood, C., Kris, M. G., Miller, V. A., Allen, J. R., Keding, S. J., Dåneshjui, S. J., Gomez, J., Tyson, L., Pizzo, B., Baetz, V., and Livingston, P. O. (2004) Vaccination of patients with small-cell lung cancer with synthetic fucosyl GM-1 conjugated to keyhole limpet hemocyanin. *Clin. Cancer Res.* 10, 6094–6100

Holmberg, L. A., and Sandmaier, B. M. (2004) Vaccination with Theratope (STN-KLH) as treatment for breast cancer. *Expert Rev. Vaccines* 3, 655–663

Sabbatini, P. J., Kudryashov, V., Ragupathi, G., Dåneshjui, S. J., Livingston, P. O., Bornmann, W., Spassova, M., Zatorski, A., Spriggs, D., Aghajanian, C., Soignet, S., Peyton, M., O’Flaherty, C., Curtin, J., and Lloyd, K. O. (2000) Immunization of ovarian cancer patients with a synthetic Lewis(y)-protein conjugate vaccine: a phase 1 trial. *Int. J. Cancer* 87, 79–85

Carcinoma and stromal enzyme activity profiles associated with breast tumor growth in vivo. *Proc. Natl. Acad. Sci. U. S. A.* 101, 13756–13761

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Kobayashi, S., Boggon, T. J., Dayaram, T., Janne, P. A., Kocher, O., Meyerson, M., Johnson, B. E., Eck, M. J., Tenen, D. G., and Halmos, B. (2005) EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* 352, 786–792

Pao, W., Miller, V. A., Politi, K. A., Riely, G. J., Somwar, R., Zakowski, M. F., Kris, M. G., and Varmus, H. (2005) Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med.* 2, 225–235

Russko, P., Loprevite, M., Cesario, A., and Ardizzoni, A. (2004) Farnesylated proteins as anticancer drug targets: from laboratory to the clinic. *Curr. Med. Chem. Anticancer Agents* 4, 123–138

Kloog, Y., and Cox, A. D. (2004) Prenyl-binding domains: potential targets for Ras inhibitors and anti-cancer drugs. *Semin. Cancer Biol.* 14, 253–261

Mo, H., and Elson, C. E. (2004) Studies of the isoprenoid-mediated inhibition of mevalonate synthesis applied to cancer chemotherapy and chemotherapy. *Exp. Biol. Med.* (Maywood) 229, 567–585

Richon, V. M., Sandhoff, T. W., Rifkind, R. A., and Marks, P. A. (2000) Histone deacetylase inhibitors for cancer chemotherapy and hemoprophylaxis. *Exptl. Biol. Med.* (Maywood) 229, 567–585

Rajkumar, S. V., Richardson, P. G., Hideshima, T., and Anderson, K. C. (2005) Proteasome inhibition as a novel therapeutic target in human cancer. *J. Clin. Oncol.* 23, 630–639

Gilewski, T., Ragupathi, G., Bhuta, S., Williams, L. J., Mussell, C., Zhang, X. F., Bornmann, W. G., Spassova, M., Bencsath, K. P., Panageas, K. S., Chin, J., Hudis, C. A., Norton, L., Houghton, A. N., Livingston, P. O., and Danielsen, S. J. (2001) Immunization of metastatic breast cancer patients with a fully synthetic globo H conjugate: a phase I trial. *Proc. Natl. Acad. Sci. U. S. A.* 98, 3270–3275

Krug, L. M., Ragupathi, G., Hood, C., Kris, M. G., Miller, V. A., Allen, J. R., Keding, S. J., Dåneshjui, S. J., Gomez, J., Tyson, L., Pizzo, B., Baetz, V., and Livingston, P. O. (2004) Vaccination of patients with small-cell lung cancer with synthetic fucosyl GM-1 conjugated to keyhole limpet hemocyanin. *Clin. Cancer Res.* 10, 6094–6100

Holmberg, L. A., and Sandmaier, B. M. (2004) Vaccination with Theratope (STN-KLH) as treatment for breast cancer. *Expert Rev. Vaccines* 3, 655–663

Sabbatini, P. J., Kudryashov, V., Ragupathi, G., Dåneshjui, S. J., Livingston, P. O., Bornmann, W., Spassova, M., Zatorski, A., Spriggs, D., Aghajanian, C., Soignet, S., Peyton, M., O’Flaherty, C., Curtin, J., and Lloyd, K. O. (2000) Immunization of ovarian cancer patients with a synthetic Lewis(y)-protein conjugate vaccine: a phase 1 trial. *Int. J. Cancer* 87, 79–85

Cohen, M. H., Johnson, J. R., Chen, Y. F., Sridhara, R., and Pazdur, R. (2005) FDA drug approval summary: erlotinib (Tarceva) tablets. *Oncologist* 10, 461–466

Ma, C., Mandrekar, S. J., Alberts, S. R., Croghan, G. A., Jatoi, A., Reid, J. M., Hanson, L. J., Bruezk, L., Tan, A. D., Pittot, H. C., Erlichman, C., Wright, J. J., and Adjei, A. A. (2006) A phase I and pharmacologic study of sequences of the proteasome inhibitor, bortezomib (PS-341, VelcadeTM), in combination with paclitaxel and carboplatin in patients with advanced malignancies. *Cancer Chemother. Pharmacol.* in press

Richards, D. A., Boehm, K. A., Waterhouse, D. M., Wagener, D. J., Krishnamurthi, S. S., Rosemurgy, A., Grove, W., Macdonald, K., Gulyas, S., Clark, M., and Dasse, K. D. (2006) Gemcitabine plus CI-994 offers no advantage over gemcitabine alone in the treatment of patients with advanced pancreatic cancer: results of a phase II randomized, double-blind, placebo-controlled, multicenter study. *Ann. Oncol.* 17, 1096–1102

Harari, P. M., and Huang, S. (2006) Radiation combined with EGFR signal inhibitors: head and neck cancer focus. *Semin. Radiat. Oncol.* 16, 38–44

David, E., Sun, S. Y., Waller, E. K., Chen, J., Khuri, F. R., and Lorial, S. (2005) The combination of the farnesyl transferase inhibitor lonafarnib and the proteasome inhibitor bortezomib induces synergistic apoptosis in human myeloma cells that is associated with down-regulation of p-AKT. *Blood* 106, 4322–4329

Kantarjian, H. M., and Cortes, J. (2006) New strategies in chronic myeloid leukemia. *Int. J. Hematol.* 83, 289–293