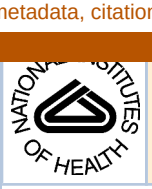

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Targeting prostate cancer based on signal transduction and cell cycle pathways

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Abstract

Prostate cancer remains a leading cause of death in men despite increased capacity to diagnose at earlier stages. After prostate cancer has become hormone independent, which often occurs after hormonal ablation therapies, it is difficult to effectively treat. Prostate cancer may arise from mutations and dysregulation of various genes involved in regulation signal transduction (e.g., PTEN, Akt, etc.,) and the cell cycle (e.g., p53, $p21^{Cip1}$, $p27^{Kip1}$, Rb, etc.,). This review focuses on the aberrant interactions of signal transduction and cell cycle genes products and how they can contribute to prostate cancer and alter therapeutic effectiveness.

Keywords

radiosensitization; prostate cancer; p53; MDM-2; MDM-2; antagonists; senescence; PTEN; Akt

Prostate Cancer: The Most Prevelant Cancer in Men

Due to increasingly reliable methods of detection, carcinoma of the prostate (CaP) has become the most commonly-diagnosed cancer in men in the United States. This year alone, approximately 230,000 American males will be diagnosed with CaP and nearly 30,000 will die from this deadly disease (source: American Cancer Society). Despite increased public awareness and improved procedures for surgical intervention of CaP, there remains no effective cure for patients with advanced disease. Moreover, treatment regimens are limited to radiation and/or chemotherapy, which primarily provide palliative benefits while offering little in the form of increased life expectancy. Consequently, studies designed to better understand the etiology of this disease should lead to more effective treatments in the clinic.

Over the past decade, scientists have discovered that early detection drastically reduces death due to CaP. Consequently, during this time a vast majority of the research that has focused on identifying prognostic indicators of advanced disease versus those markers associated with

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earlier stages of CaP. However, much less has been discovered during this time regarding the development and natural progression of the disease.

There are a number of factors that have been correlated to the development of CaP including age (increased diagnosis after 60 years; 1 in 7 men), environmental factors (race, diet, etc.,), familial inheritance (10% of CaP are associated with hereditary factors), and steroid hormone signaling (decreased reliance upon testosterone), among others.[1] While each of these factors appears to be intimately associated with the development of disease, one must comprehend what occurs on the molecular level to develop better treatment strategies and possible cures.[2,3]

The prostate gland surrounds the urethra at the base of the bladder and functions by producing secretory proteins for semen. There are at least three different cell types that comprise the prostatic epithelium: (1) secretory luminal epithelial cells—a well-differentiated androgen-dependent cell that functions to secrete prostatic proteins, (2) basal cells—situated between the secretory luminal cell layer and the underlying basement membrane, (3) neuroendocrine cells—androgen-independent cells that are located in the same stratum as the basal cells and are believed to provide paracrine signals to the adjacent luminal cells, and (4) prostate stem cells—not proven yet, but anticipated to exist.

Prostatic intraepithelial neoplasia (PIN) are preneoplastic lesions that precede the advent of prostate cancer. PIN lesions are characterized by loss of basal cells and invasion of luminal cells into the periphery. The exact causes surrounding the onset of PIN are unknown; however, a prominent theory is that loss of particular chromosomes may be major contributory factors in this process. Chromosomes 8p, 10q and 13q are all frequently lost in CaP; there are a number of important tumor suppressor genes found on these chromosomes including NKX3.1,[4-6] PTEN/MMAC1,[7-12] and Rb,[13-16] respectively.

Central Role of PI3K/PTEN/Akt/mTOR Pathway in Prostate Cancer

Chromosome 10q is frequently lost (50-80%) in prostatic lesions. PTEN is located within this locus (10q23) and is a well-known negative regulator of the PI3K/Akt signal transduction cascade. The PI3K/Akt signaling pathway is known for its role in mediating cell survival, as well as cell cycle progression and neoplastic transformation.[17] Consequently, it would stand to reason that mutation of PTEN would initiate heightened activation of this cascade, which could then confer a number of cancer-like properties to the cell. Many of these, particularly cellular survival, are hallmark features of CaP tumor cells.

Phosphatidylinositol-3-kinase (PI3K) is a heterodimeric protein with an 85-kDa regulatory subunit and a 110-kDacatalytic subunit. PI3K serves to phosphorylate a series of membrane phospholipids including PtdIns(4)P and PtdIns(4,5)P_2, catalyzing the transfer of ATP-derived phosphate to the D-3 position of the inositol ring of membrane phosphoinositides, thereby forming the second messenger lipids PtdIns(3,4)P_2 and PtdIns(3,4,5)P3. Most often, PI3K is activated via the binding of a ligand to its cognate receptor, whereby p85 associates with phosphorylated tyrosine residues on the receptor via an SH2 (Src-homology 2) domain. After association with the receptor, the p110 catalytic subunit then transfers phosphate groups to the aforementioned membrane phospholipids. It is these lipids, specifically PtdIns(3,4,5)P_3, that attract a series of kinases to the plasma membrane thereby initiating the signaling cascade.[18] An overview of the PI3K/PTEN/Akt/mTOR pathway is presented in Figure 1.

Downstream of PI3K is the primary effector molecule of the PI3K signaling cascade, Akt/PKB (protein kinase B). Akt was originally discovered as the cellular homologue of the transforming retrovirus AKT8 and as a kinase with properties similar to protein kinases A and C.[19,20] Akt contains an amino-terminal pleckstrin homology (PH) domain that serves to target the protein

to the membrane for activation.[21-23] Within its central region, Akt has a large kinase domain and is flanked on the carboxy-terminus by hydrophobic and proline-rich regions.[24,25] Akt is activated via phosphorylation of two residues: T308, S473. While it is generally agreed upon that maximal activation of Akt requires phosphorylation of both of these residues, there are conflicting reports regarding the relative importance of S473 and T308 for initial activation of Akt. It was originally thought that either of these residues could be phosphorylated to initiate Akt activity and that phosphorylation of one residue was not necessary for phosphorylation of the other to occur.[26] More recent evidence suggests that phosphorylation of T308 is absolutely essential for phosphorylation of S473.[27,28] Each of these studies was carried out in the HEK293 cell line, which further compounds the issue because the differences cannot be attributed to cell type.

The phosphotidylinositide-dependent kinases (PDKs) are responsible for activation of Akt. PDK1 is the kinase responsible for phosphorylation of T308,[29] while the actual kinase that phosphorylates S473 (PDK2) remains controversial. Some report that phosphorylation of T308 triggers autophosphorylation of S473,[30] while others hypothesize that PDK2 is the integrin-linked kinase (ILK).[31] However, it is now believed that ILK may facilitate phosphor-ylation of S473 indirectly, perhaps by acting as a scaffolding protein.[32] Akt can also be phosphorylated by the mTOR complex called Rictor.[33] Phosphorylation of Akt is complicated as it can be phosphorylated by a complex which lies downstream of itself. Moreover, it can be dephosphorylated by $p70^{S6K}$ which also lies downstream of Akt.[34] Once activated on either/both of these residues, Akt leaves the cell membrane to phosphorylate intracellular substrates.

After activation, Akt is able to translocate to the nucleus[35] where it affects the activity of a number of transcriptional regulators. Cyclic-AMP response element binding protein (CREB),[36] E2F,[37-39] NFκB (via Iκ-K),[40,41] and the forkhead transcription factors[42-50] are all either direct or indirect substrates of Akt and each can promote either cellular proliferation or survival. Aside from transcription factors, Akt is able to target a number of other molecules to affect the survival state of the cell including caspase-9,[51] the pro-apoptotic molecule BAD,[52,53] and glycogen-synthase kinase-3_β (GSK-3β).[54] When these targets are phosphorylated by Akt, they may either be activated or inactivated but the end result is to promote survival of the cell.

Negative regulation of the PI3K pathway is primarily accomplished through the action of the PTEN tumor suppressor protein. *PTEN* encodes a lipid and protein phosphatase whose primary lipid substrate is PtdIns(3,4,5)P_3. The protein substrate(s) of *PTEN* are more varied, including focal adhesion kinase (FAK), the Shc exchange protein and the transcriptional regulators ETS-2,[55] and Sp1.[56] PTEN also may negatively regulate the activation of the platelet-derived growth factor (PDGF) receptor.[57]

PTEN has four primary structural domains. On the amino terminus is the lipid and protein phosphatase domain, which is flanked adjacent to the C2 domain that is responsible for lipid binding and membrane localization. Next are two PEST domains which regulate protein stability. Lastly, PTEN has a PDZ domain, which helps facilitate protein-protein interactions. Mutations within the phosphatase domain have been reported to nullify the endogenous function of PTEN.[58-60]

As previously mentioned, loss of PTEN function in advanced CaP is quite common (50-80% of patients).[61,62] As a consequence, this results in an overabundance of lipid second messengers [PtdIns(3,4,5)P_3], which can cause constitutive activation of PH domain-containing proteins including Akt. It is for this reason that Akt is found to be highly-activated in advanced cases of CaP.[63,64] Aberrant Akt activation is able to elicit the pro-survival properties observed in CaP cells through a number of mechanisms, described hereafter. Hence inhibiting Akt or restoring PTEN activities are potential therapeutic targets in prostate cancer.

$p27^{Kip1}$ is a central mediator of cell cycle progression whose role as a cyclin-dependent kinase (CDK) inhibitor is lost in many cases of advanced CaPs.[65-69] The role of $p27^{Kip1}$ in preventing prostatic disease is bolstered by evidence which shows that $p27^{Kip1}$-null mice quickly develop prostatic hyperplasia.[70] Akt appears to affect $p27^{Kip1}$ expression via the intermediate molecule, FKHR. FKHR (FOXO1) is a member of the forkhead/FoxO family of transcription factors, which are known to stimulate transcription of $p27^{Kip1}$.[71] Upon activation, Akt phosphorylates FKHR causing its inactivation and subsequent downregulation of $p27^{Kip1}$. Further evidence has shown that constitutively-active Akt may be able to decrease the half-life of $p27^{Kip1}$ as well.[72,73] This series of events leads to a phenotype whereby CaP cells have less restriction on cell cycle progression, thereby promoting unregulated cell division. Supporting these observations are other studies which show that as CaP progresses from an androgen-dependent to -independent state, $p27^{Kip1}$ levels are drastically diminished.[74,75]

Akt regulates the apoptotic response to a variety of stimuli via its ability to interact with a number of key players in the apoptotic process. First, Akt can directly phosphorylate BAD on S136,[76] causing its inactivation and inability to interact with anti-apoptotic members of the Bcl-2 family of proteins (Bcl-2, Bcl-X_L).[77] Next, activated Akt can independently inhibit the release of cytochrome c from the mitochondria, which is a potent activator of the apoptotic caspase cascade.[65] Akt also is capable of phosphorylating procaspase-9, preventing its cleavage into the pro-apoptotic caspase-9 initiator of programmed cell death.[51] Lastly, the Akt target, FKHR is capable of upregulating Fas ligand and Bim, two very important molecules that are potent inducers of apoptosis; however, when inactivated by Akt, FKHR is localized to the cytosol where it is unable to augment expression of these genes.[49,79] Akt can also phosphorylate Bim which inhibits its proapoptotic activity. In concert, these events caused by Akt activation would appear to greatly affect the survival status of the cell.

Akt also plays significant roles in protein translation, particularly by regulating those proteins involved in growth and survival. A specific target is mTOR (mammalian target of rapamycin) which is able to induce phosphorylation of eIF-4E binding protein-1 (4E-BP1). After the appropriate phosphorylation events, 4E-BP1 disassociates from the mRNA cap-binding protein eIF-4E, which allows eIF-4E to interact with the eIF-4E translation initiation complex to initiate protein synthesis. Another target of Akt is $p70^{S6K}$ which is a well-known enhancer of protein synthesis.[80-84] mTOR has been shown to be critically important in autophagy,[85-87] a mechanism of cell death which will be discussed below.

Messenger RNAs differ in their ability to be translated; the length and sequence of the 5' UTR largely dictates the efficiency with which an mRNA transcript will be translated. Most mRNAs contain short, unstructured GC-poor 5' UTRs and are efficiently translated. In contrast, protooncogene and growth factor mRNAs are characterized by long, GC-rich sequences in the 5' UTR, which can often hinder the ability of the eIF-4E complex to efficiently scan and initiate translation at the start codon. Consequently, under normal circumstances these mRNAs are not efficiently translated.[88,89] However, upon Akt-mediated activation of mTOR, these latter mRNAs are highly and disproportionately translated; several key proteins that are overexpressed as a consequence of this event include c-myc,[90-96] cyclin D1,[97,98] and VEGF[99] among others.[88,89] Cyclin D1 has been reported to be overexpressed in CaP xenografts and metastases,[100,101] while early stage prostatic lesions possess much lower levels of the protein.[102] A number of reports support the notion that mTOR signaling is a prominent feature of CaP progression, as recurrent tumors have altered expression of a number of molecular targets of rapamycin.[103-107] Hence mTOR inhibitors such as rapamycin may be effective in prostate cancer therapy.

Role of MAPK Signaling in Prostate Cancer

The PI3K/Akt signaling cascade is primarily associated with cell survival; conversely, the Raf/MEK/ERK pathway is associated with growth, proliferation and differentiation.[108-114] Despite the wealth of information surrounding the role of PI3K-mediated signaling in prostate cancer, much less is known about the function of Raf/MEK/ERK signaling in CaP.

Like PI3K, activation of the Raf/MEK/ERK pathway is initiated by a mitogen-receptor ligation at the cellular surface. Growth factor receptors are comprised of at least two subunits which oligomerize upon binding of the respective mitogen; this event is immediately preceded by increased tyrosine kinase activity on the cytoplasmic portion of the receptor. Most often this is due to autophosphorylation, whereby one subunit of the receptor acts as a tyrosine kinase and another subunit serves as a substrate. The phosphorylated tyrosine residue(s) then serve as docking sites for cytoplasmic proteins that contain SH2 domains.[115] After this event, a complex of proteins is able to initiate a series of phospho-relay events that can ultimately alter gene expression in the nucleus, as described hereafter.

Ras is a 21-kDa membrane-localized, monomeric G-protein that is considered to be the initiator of signaling in the Raf/MEK/ERK pathway. The consensus amongst researchers is that mutations in the Ras gene are relatively uncommon in prostatic malignancies[116-118] as compared to other tumor types (~30%). After receptor ligation and activation, SH2-containing proteins are targeted to the phosphorylated tyrosine residue(s) on the receptor; one such protein is Grb2.[119] Grb2 also contains an SH3 domain whose primary function is to bind to proline-rich regions of other molecules, such as SOS (Son of Sevenless). SOS is a guanine dissociation stimulator of Ras which binds to Ras after receptor activation. After binding to SOS, Ras undergoes a conformational change that causes the dissociation of GDP from Ras, allowing it to then associate with GTP. In the GTP-bound form, Ras is in its active state and able to recruit the Raf kinase to the plasma membrane for activation.[120-123] To negatively regulate this process, there exist a number of GTPase-activating proteins (GAPs) that are able to stimulate the GTPase activity of Ras in order to convert it back to its original GDP-bound state.[124]

Immediately downstream of Ras is the Raf kinase. Currently, there are three known isoforms of Raf: Raf-1 (c-Raf), A-Raf and B-Raf.[125-128] Each of these isoforms has been described to have both overlapping and unique regulatory functions.[128] Activation of Raf (A-Raf and Raf-1 but not B-Raf) is a two-step process—first, active GTP-bound Ras recruits Raf to the cellular membrane, which allows association with additional proteins necessary for activation (i.e., scaffolding proteins, etc.,).[129] The first step is necessary but not sufficient for activation of Raf. Consequently, a second step is required whereby Raf (A-Raf and Raf-1 but not B-Raf) is phosphorylated on tyrosine and/or serine/ threonine residues. A member of the Src family of kinases is responsible for this event when it occurs on tyrosine residues,[130] whereas protein kinase C (PKC) is a kinase implicated in activating Raf on serine/threonine residues.[131,132] An overview of the Raf/MEK/ERK pathway is presented in Figure 2.

Regulation of Raf activation, however, is much more complex than simple phosphorylation of a few nominal residues. Dr. Walter Kolch has contributed significantly to this area and has published a number of thorough reviews on the topic.[133-136] Briefly, Raf-1 is maintained in an inactive state when phosphorylated on serines 259 and 621 by binding to 14-3-3 proteins.[136-138] Activation of Raf-1 is initialized when Ras displaces the 14-3-3 protein, allowing phosphatase 2A (PP2A) to access and remove the phosphate group from S259.[139,140] Evidence suggests that nearly 80% of all Raf-1 exists in the phospho-S259 form and that it is impossible to activate the protein while in this state.[138,141] When activated, Raf-1 is phosphorylated on S338; mutation of this residue is sufficient to eradicate Raf-1 activation by mitogenic stimulation.[142,143] For these reasons, the use of phosphospecific antibodies to

detect Raf-1 when phosphorylated on S338 has been used extensively as a qualitative indicator of Raf-1 activity with reliable accuracy.[143]

After activation, Raf targets its downstream substrate(s) MEK1/2. MEK-1 and MEK-2 (hereafter, MEK) are phosphorylated on S218 and S222 by the Raf proteins.[144] Phosphorylation on these two residues has been reported to increase MEK activity over 7000-fold.[145] The kinase suppressor of Ras (KSR) is a scaffolding protein that mediates the interaction between Raf and MEK, allowing for efficient phosphorylation of the MEK protein.[146] Recent reports have suggested that the association of Raf, MEK and KSR is not as simplistic as it was once thought—there appear to be modulators of KSR activity, including MEK partner-1 (MP1)[147] and Raf kinase inhibitor protein (RKIP).[148] The function of MP1 is believed to be another scaffolding protein that enhances the association of MEK and B-Raf, whereas RKIP actually disrupts the binding between Raf-1, MEK and RKIP. Together, each of these proteins plays a unique role in the activation of MEK by Raf although it appears that the molecules involved may be more numerous than just these proteins.[133] RKIP has been postulated to be a tumor suppressor protein. Relatedly, decreased expression of RKIP has been associated with prostate cancer progression.[148-150]

ERK-1 and -2 are 44 and 42 kDa kinases that are directly downstream of the MEKs. Phosphorylation of ERK-2 occurs on residues T183 and Y185; maximal activation is achieved via phosphorylation by MEK on both of these residues.[151,152] After activation, ERK-2 can dimerize with other ERK-2 molecules and induce nuclear translocation.[153] Upon entering the nucleus, ERK-2 dimers are thought to activate an array of nuclear targets, including topoisomerase II-α, suggesting that ERK-2 has a role in chromatin remodeling during mitotic events.[154] A number of cytoplasmic ERK targets exist as well, the primary of which is the p90 ribosomal S6 kinase ($p90^{RSK}$)[155] which is responsible for phosphorylating several transcriptional regulators including CREB,[156] estrogen receptor-α[157] IκB/NFκB,[158,159] and c-Fos.[160] In addition to $p90^{RSK}$, ERK is also a known inducer of the activator protein-1 (AP-1) family of transcription factors; these modulators of transcription include c-Jun, c-Fos and ATF-1 and each alters gene expression of individual target genes when activated. Each of the above described phosphorylation promotes an environment that favors proliferation.

MAPK signal transduction has not been extensively studied in the prostate; very few publications exist detailing the involvement of the Raf/MEK/ERK pathway in the advancement of prostatic disease. Within the data that have been reported, there exists some degree of divergence—some reports show that this cascade is involved in CaP development, while others report the opposite. Consequently, the current level of understanding of MAPK activation in CaP is ambiguous.

While aberrant MAPK activity is common in many cancers, there is little evidence to suggest that MAPK activity is increased in CaP. The absence of mutations in Ras may be a factor that contributes to this observation,[117,118] as many cancers with constitutive MAPK activity possess mutations in the Ras G-protein. One study has reported that activation of the MAPK pathway with conditionallyactivated mutants of Raf resulted in cell cycle arrest and decreased plating efficiency.[161] Moreover, the authors suggested that activation of this pathway may be a therapeutic target for CaP cells. Supporting this notion are other studies that show that treatment of CaP cells with resveratrol or phenylethyl isothiocyanate, two compounds that induce apoptosis in a variety of cancers, can induce apoptosis in cells of prostatic origin in an ERK-dependent manner.[162-164]

The use of ERK activation as a prognostic indicator of disease is quickly becoming a useful tool in determining the stage of progression of CaP. Initially, it was reported that ERK activation was increased in high-grade PIN when compared to tissue derived from the normal

prostate, as measured by immunohistochemical analysis.[165,166] Another study supports this observation; Price et al., show that there was significantly increased activation of ERK in tissue removed from patients who had undergone radical prostatectomies.[167] Contrary to these results, however, are other reports which state that ERK activation is actually diminished as the disease progresses from normal tissue to PIN to invasive carcinoma.[168,169] Taken together, these data are non-conclusive, but they do suggest that ERK activity may be a viable means of determining patient prognosis if accurately ascertained. ERK activity may be increased in certain subsets of prostate cancer patients.

Roles of Cell Cycle Proteins in Prostate Cancer

In addition to the PI3K/PTEN/Akt and Raf/MEK/ERK and other signaling pathways, lies a complex network of proteins which regulate cell cycle progression. Often some of these cell cycle regulatory proteins (e.g., p53) may serve to regulate the activity of signaling pathways including PI3K/PTEN/Akt and Raf/MEK/ERK.[170-181] An overview of some of these potential interactions is presented in Figure 3. Numerous proteins involved in cell cycle regulation are often mutated in prostate cancer, resulting in activation of oncogenes and loss of tumor suppressor proteins. There is no dominant molecular profile that initiates or maintains prostatic carcinogenesis, but rather a complex mixture of mutations that disrupt cell cycle control and cell death pathways. Cell cycle proteins commonly mutated at early to late stages of prostate cancer progression include Rb, p14, p16, p53 and p27.[182-189] Mutations within these proteins result in defective cell cycle checkpoint control, leading to further chromosomal instability and inactivation of the protective tumor preventative failsafe, cellular senescence.[190-193] The PI3K/PTEN/Akt and Raf/MEK/ERK pathways are also commonly the target of mutations that promote cell proliferation and block apoptosis and elicit some of their effects through cell cycle pathways. Furthermore, and perhaps more importantly mutations at upstream receptor genes feed into these pathways. Combined mutations within and among these pathways may alter the success of radio- and chemotherapies. Knowledge of the pathways altered, prior to initiating therapy, could provide a molecular signature for clinicians to optimize sensitization strategies based upon the individual's profile. The use of small-molecule inhibitors aimed at dysregulated pathways in combination with various therapeutic approaches may improve the success of treatment and prolong survival of the patient.[194-203]

Although the frequency of p53 mutations in early prostate cancer is low, heterozygous loss of function (LOF) mutations often accompany late stage carcinoma.[189,204] Current models of the molecular network coordinating responses to DNA damage place p53 at the crossroads of several stress response pathways critical for maintaining genome integrity; including cell cycle arrest, DNA repair, mitotic catastrophe, apoptosis and cellular senescence.[205-211] The mechanisms by which p53 directs cells down each of these alternative avenues of cell fate determination differs among cells of various origins.[212-214] It is important that each of these distinct forms of p53-dependent cell death be collectively evaluated when investigating new therapeutic strategies for increasing the sensitivity of CaP cells to various therapeutic approaches.

Since p53 function is crucial to coordinating the DNA damage response, multiple upstream pathways control p53 activation. While the list of stress signals that activate p53 continues to grow, including hypoxia, ribosomal stress and loss of cell-cell contacts, the primary pathways appear to be oncogenic stress and DNA damage. Interestingly, various stress signals use different mechanisms for p53 activation. Under resting conditions, the ubiquitin degradation pathway maintains a high rate of p53 turnover that is dependent on the E3 ubiquitin ligase, murine double-minute 2 (MDM2).[215-218] Hypophosphorylated MDM2 binds the p53 C-terminus and targets p53 for degradation.[219] Moreover, the p53 binding domain of MDM2 overlaps with the DNA binding domain of p53, effectively impairing p53 activity.[220] The p53

protein is also regulated by MDMX, a homologue of MDM2, that binds MDM2 through its C-terminal RING domain and stimulates the MDM2-dependent ubiqutination of p53.[215,221,222] Therapy induced DNA damage triggers a phosphorylation cascade involving ataxia teleangetasia mutated (ATM) and ATM-related (ATR) proteins that prevent p53 degradation through multiple phosphorylation events on MDM2, MDMX and p53.[223-228]

Active p53 functions as a transcriptional regulator within a complex network involving the selective transactivation and transrepression of multiple genes involved in cell cycle control and apoptosis. p53 functions as a tetrameric transcription factor. The hetero-oligomerization between a heterozygous mutant allele and wild-type (WT) p53 proteins may be sufficient to alter the function of p53 tetramers. The dominant negative (DN) actions of missense *p53* mutations were first demonstrated in experiments with ectopic expression of WT and mutant p53 proteins[204,229] and more recently in thymocytes expressing *p53* point mutations at single-copy levels.[230,231] Alternatively, mutant *p53* alleles may impart a gain of function (GOF) that could also impact tumor development and the progression of prostate cancer.[230-233]

p53 can activate the Raf/MEK/ERK pathway by the discoidin domain receptor-1 (DDR-1).[234-236] Furthermore, ERK can phosphorylate p53 which results in its stabilization.[237] The PI3K/PTEN/Akt pathway also serves to regulate p53 activity through Akt-mediated phosphorylation of MDM-2 which enhances its activity and destabilizes p53.[238] Furthermore, there are reciprocal interactions between p53 and PTEN which serve to cross regulate each other's expression.[239-242] Thus targeting the p53, PI3K/PTEN/Akt/mTOR and Raf/MEK/ERK pathway may enhance certain prostate cancer therapies.

Many studies of in vitro prostate cancer sensitivity have used apoptosis assays to assess the efficacy of cell killing and have been limited to a few CaP cell lines that were derived from metastatic lesions containing p53 mutations rarely observed in organ-confined tumors.[233,243-245] The best characterized lines include PC-3 and DU145 cells that harbor pairs of deleted or inactivated p53 alleles and display a complete loss of p53 function, a condition often associated with decreased radiosensitivity.[246-248] However, in cultures of CaP cells retaining a functional p53 protein (LNCaP and 22Rv1) cellular senescence is the dominant form of death. Alternative stress response pathways controlled by this tumor suppressor, including cell cycle arrest, DNA damage repair, mitotic catastrophe and apoptosis, contributed significantly less to radiation-induced death.[249] Although autophagy is an additional form of cell death that has recently been reported to enhance the therapeutic sensitivity of PC-3 and DU145 cells,[249] neither of these cell lines express a functional p53. Thus, in the contemporary setting of earlier detection where complete p53 inactivation is rare, the induction of terminal growth arrest may be a primary mode of clonogenic death in radiation therapy of prostate cancer.[250] This would be consistent with the observation that in some patients complete regression of prostatic tumors can take more than a year after finishing therapy.

Recent evidence indicates that tumor regression after therapy might involve the phagocytosis of senescent cells by macrophages and the autophagy of senescent cells themselves. Pronounced alterations in the endosomal/lysosomal pathway are evident in senescent cells. Lipofuscin accumulation at the level of autophagic vacuoles accounts for the increased granularity observed in the side-scatter profile of senescent cells and is a hallmark of cellular senescence. Autophagy is an ancient pathway for homeostatic turnover of long-lived intracellular components and for nutrient acquisition during starvation and stress. Recently, p53 has been shown to positively modulate damage-regulated autophagy modulator (DRAM)-dependent autophagy.[251] Thus, therapy induced activation of p53, cellular senescence and autophagy may contribute to the tumor regression observed in vivo when investigators have experimentally “turned on” the *p53* gene in tumor-bearing mice.[252,253]

The p53, p21 and Rb tumor suppressors are important senescence regulators. p53, p21 and Rb are transiently activated in response to stressors that induce 'premature' senescence in tumor cells. Transactivation of the cyclin-dependent kinase (CDK) inhibitor p21 appears most relevant as a downstream mediator of p53-dependent terminal growth arrest.[254] How p21 and other CDK inhibitors (e.g., $p16^{Ink4a}$) promote senescence is not precisely known, but one well-studied mechanism for p21-induced senescence involves the activation of Rb. Rb family members (Rb, p107, p130) are corepressors of the E2F transcription factors required for cell proliferation. With an accumulation of CDK inhibitors and the onset of senescent arrest, Rb is converted to an active hypophosphorylated form that stably interacts with, and sequesters, E2F and other proteins that influence gene expression and promote cell cycle progression.[255] Notably, the cellular levels of p53, p21 and active Rb do not remain elevated after the onset of senescence, suggesting that molecular interactions required to initiate the senescent state may be dispensable for its maintenance. For example, conditional expression of p53, p21 or p16 causes cellular senescence in many settings, and the cells remain in a senescent state after promoter shutoff.[256-258] Because expression of another CDK inhibitor, p16, rises as p21 goes down, it has been suggested that p16 may be responsible for maintaining a stable growth arrest, while others contend that both p53 and p16 function in the maintenance of growth arrest in senescent cells.[259]

Another recent explanation for the permanence of cellular senescence is that p16 enables Rb to establish heterochromatin changes that maintain E2F-responsive promoters in an irreversibly silenced state and no longer dependent on the presence of p16 or Rb.[255] These unusual foci of heterochromatin are physically associated with tightly packed and trimethylated histone proteins (e.g., H3K9) and the histone methyltransferase Suv39h1.[260,261] Whether these p16/Rb-initiated modifications of heterochromatin are truly irreversible remains to be determined. Moreover, there is evidence that the premature senescence induced by chemotherapeutic drugs and radiation in tumor cells can occur in the absence of p53, p21 as well as p16, indicating that additional genes and pathways may also mediate damage-induced senescence of tumor cells.[262]

Rb inactivation is common in prostate cancer and generally precedes somatic alterations affecting the tumor suppressor genes *p53* and *PTEN*.[183,185,263] Interactions among these three major tumor suppressor genes appear to directly influence tumor development in the mouse prostate.[264,265] For instance, conditional deletion of an *Rb* allele specifically in the mouse prostate epithelium results in the development of focal hyperplasia but not tumorgenesis,[266] while acute inactivation of *PTEN* alone induces a p53-dependent cellular senescence.[264] In contrast, when combined with inactivation of the Rb family proteins (Rb/p107/p130) or *p53*, a loss of *PTEN* either elicits an accelerated rate of tumor progression or development of invasive adenocarcinoma in mice, respectively.[264-266] These transgenic studies establish that a p53-mediated senescence response restricts cell proliferation after loss of *PTEN* in the mouse prostate in vivo and that a concomitant loss of either *Rb* or *p53* is sufficient to bypass this impediment to tumor growth.

Treatment of Prostate Cancer

The primary treatment strategy for non-metastatic CaP is generally either radiation or complete removal of the prostate (radical prostatectomy). Unfortunately, patients often relapse and aberrant growth of the prostate returns (or the neoplasia is not detected in its infantile stages). These patients are most often subjected to androgen ablation therapy in which either chemical or surgical castration is performed in concert with non-steroidal anti-androgens. Furthermore, since prostate cells are heavily reliant upon androgens for growth and/or survival, these cells generally die within a short period of time. Consequently, the prostatic tumor undergoes a complete remission for a period of approximately 18 months[267]

After androgen ablation therapy, most tumors eventually relapse and begin to grow in the absence of testosterone; this event is known as hormone relapse and patients in this stage are said to possess hormone-refractory prostate cancer (HRPC). The expected lifespan of patients who progress to the point of HRPC is less than 24 months and the prognosis is invariably death.[267] Hormone relapse is almost always accompanied by an increase in prostate-specific antigen (PSA) levels. It is for this reason that measurement of PSA levels has become the primary means of detection for tumorigenicity within the prostate.

After hormone relapse, therapeutic options become more limited. Modern chemotherapy is just beginning to be commonly-utilized in the clinic. Until recently, it was believed that chemotherapy was relatively ineffective against CaP cells,[268,269] however, the development of the PSA test as a qualitative measurement of therapeutic efficacy soon refuted the notion that chemotherapy was ineffective in tumor cells of prostatic origin. Before the PSA test, efficacy of chemotoxic drugs was primarily monitored by more objective means (tumor size and/or volume), however it has now become evident that this method of assessing drug efficacy is both inaccurate and unreliable—reported response rates from the same drug were increased from 20% (tumor size) to nearly 75% (PSA levels) merely by changing the detection method used in these studies.[199] In light of these discoveries, there is a renewed interest in determining the therapeutic value of chemo-therapy in CaP patients.

Most often, CaP patients are administered a variety of chemo-therapeutic compounds to combat the disease.[270-275] Employing the use of these drugs, however, has been met with only modest success due to issues of toxicity and the drug-resistant nature of CaP. Efforts to minimize drug resistance should prove effective in extending the life expectancy of HRPC patients.

Drug resistance is manifested via a multitude of biomolecular events including inhibiting entrance of chemotoxic agents into the cell, loss of enzymatic activities involved in metabolizing the drug, and overexpression of ABC (ATP-Binding Cassette) drug efflux pumps, among others.[276-278] Recently, we and others have shown that aberrant activity of the PI3K/PTEN/Akt/mTOR signaling cascade can augment drug resistance in advanced CaP cells.[279,280] These reports detail several potential mechanisms responsible for increased chemoresistance including PI3K-induced overexpression of the MRP-1 drug pump as well as activation of the mTOR molecule that is downstream of PI3K/Akt. Consequently, targeted inhibition of the PI3K/Akt signaling pathway is quickly gaining interest with regards to chemotherapeutic intervention in an array of cell types[281,282]

Chemotherapy of Prostate Cancer

Chemotherapy agents used to combat CaP usually fall into three categories: (1) interchelating agents (2) microtubule stabilizing agents, and (3) alkylating compounds. Anthracyclines are antibiotic compounds derived from the *Streptomyces* species that are used to treat a variety of cancers including CaP. Daunorubicin, doxorubicin and epirubicin are all members of this class of compounds. Anthracyclines possess a number of mechanisms of action including intercalating between base pairs of DNA, creating free radicals that initiate DNA damage, and inhibition of the nuclear enzyme topoisomerase II.[283] By intercalating into DNA, anthracyclines inhibit both DNA and RNA synthesis.[284,285] Inhibition of topoisomerase II, which causes torsional strain on DNA causing strand breakage, was later proven to be an integral event in anthracycline-induced cell death.[286] Together, these modes of action initiate a series of events that can ultimately lead to the demise of a cancer cell

Mitoxantrone is an anthracenedione with a structure similar to doxorubicin but appears to have a better toxicity profile than that of anthracyclines.[287,288] Due to its similar structure to anthracyclines, it is not surprising that the mechanism of action of mitoxantrone is similar as well. Mitoxantrone is thought to induce apoptosis by both DNA intercalation and inhibition of

topoisomerase II activity.[287,288] Mitoxantrone has been investigated for its ability to induce death of CaP cells—these studies have concluded that mitoxantrone is effective in palliation of CaP symptoms, but is unlikely to prolong survival.[287,288]

The central premise behind all neoplasia is the uncontrolled nature of cell division. An accumulation of genetic events (i.e., mutations) that affect the cell cycle can lead to aberrant growth and cell division. Therefore, the process of mitosis whereby the mitotic spindle functions to divide chromatids to each daughter cell is a viable target in many cancers. Microtubules comprise the mitotic spindle and proper polymerization/depolymerization of these microtubules is essential for efficient progression through mitosis and subsequent cell division. Taxanes are a family of chemotherapeutic drugs that have the capacity to bind to microtubules, specifically β-tubulin, thereby preventing depolymerization.[289,290] This event is essential for cell division; as such, the use of taxanes has become prominent in cancer research, particularly CaP.[291-293] There are a growing number of taxanes currently available in the clinic including paclitaxel and docetaxel. Interestingly, these drugs may have an additional modes of action: they appear to induce the phosphorylation and inactivation of the anti-apoptotic molecule, Bcl-2.[294,295]

Estramustine is a stable conjugate of estradiol and nornitrogen mustard[296] that is commonly used in CaP patients. Estramustine was designed as an alkylating agent specifically for treatment in tumors of prostatic origin. The premise of the initial design of the drug was that the estradiol portion of the agent would facilitate uptake by steroid receptors, whereas the nitrogen mustard moiety would perform the alkylation; however, evidence exists to refute the notion that estramustine acts in this manner. It is thought that estramustine inhibits microtubule function and affects structural proteins within the nuclear matrix.[297,298] In high doses, estramustine is not well-tolerated,[299] however it is currently being evaluated in combination with other drugs at less toxic doses and results have been promising.

Roles of PI3K/PTEN/Akt/mTOR, Raf/MEK/ERK and p53 Pathways in Prostate Cancer Drug Resistance

The PI3K/PTEN/Akt/mTOR signal transduction pathway is well-known for its roles in cell cycle progression, survival and growth of cells. Recent evidence suggests that this pathway may also contribute to the development of drug resistance in a variety of cell types.[300-308] Efforts to identify specific molecules that confer drug resistance will be the next wave of these types of studies. To this point, the data disproportionately implicates the PI3K/PTEN/Akt/mTOR pathway in the development of drug resistance. However, this pathway is capable of inducing such effects in a number of ways; as such, it is necessary to investigate potential means of resistance in these cell types.

Given the involvement of ABC drug transporters in acquired chemoresistance, we monitored the expression of prototypical members of the ABC superfamily of efflux pumps.[281] None of the CaP cell lines tested (LNCaP, 22Rv-1, DU145 or PC3) was positive for *MDR-1* gene expression. Interestingly, CaP cells with constitutive PI3K activity were positive for *MRP-1* expression, whereas PTEN-positive (DU145 wild-type) cells expressed minimal levels of this gene.[281] However, activation of PI3K in DU145 cells potentiated upregulation of *MRP-1*, indicating that the PI3K pathway may modulate expression of the *MRP-1* gene. The observations made on the RNA level were congruent with those reported in a recent publication where the authors showed that MRP-1, not MDR-1, was the predominant drug pump expressed in advanced CaP cells.[281] These trends were also observed on the protein level when various DN PTEN and constitutively-active PI3K mutants were observed to increase levels of the *MRP-1* gene product.[281] Collectively, the data identify MRP-1 as a mediator of CaP drug resistance and an indirect mechanism responsible for its upregulation.

Wang and Beck were the first to hypothesize that p53 is a negative regulator of *MRP-1* expression.[309] However, to date, there is no report of any p53-binding motifs located within the *MRP-1* promoter region.[310] Therefore, one might hypothesize that repression of *MRP-1* by p53 occurs by an indirect means rather than an immediate interaction with the *MRP-1* promoter. Beck also reported that expression of the specificity protein-1 (Sp1) transcription factor is a strong activator of *MRP-1* expression.[309] Given that there are three Sp1 binding sites in the *MRP-1* promoter and that Sp1 activity has been reported to be PI3K-dependent,[310] it is plausible to suggest that Sp1 is critical for *MRP-1* expression in advanced CaP cells. p53 (LNCaP: p53-positive, DU145 and PC3: p53-negative) may be the dominant regulatory molecule governing MRP-1 expression, but PI3K activity and subsequent Sp1 activation, may be necessary for maximal expression of *MRP-1*.

To assess the role of MRP-1 in mediating chemoresistance in DU145 and PC3 cells, RNA interference was exploited to inhibit expression of this gene. The results presented in Lee et al., prove that inhibition of MRP-1 function, through downregulation of *MRP-1* expression, can sensitize *MRP-1*-expressing cells to chemo-therapeutic compounds.[281] The importance of these findings is significant because it provides a viable target for future antineo-plastic drug formulations—agents synthesized to antagonize the activity of MRP-1 should increase the efficacy of chemotoxic drugs while having minor, if any, effects on the viability of cells not expressing *MRP-1*.

Lastly, it is imperative to consider the context of PI3K signaling in CaP cells. The PI3K/PTEN/Akt/mTOR pathway is most often accepted as a mediator of cellular survival, rather than proliferation (i.e., MAPK signaling). Within the prostate gland, the population of cells that undergo active proliferation is between 1-3%[311,312] and this observation may explain why diagnosis of CaP does not often occur before the latter stages of life. Although proliferation of CaP cells is slow, turnover of CaP cells appears to be slower—the loss of PTEN and upregulation of PI3K activity leads to a pheno-type whereby the cell does not undergo apoptosis when directed. Consequently, pathways that mediate survival, rather than proliferation (i.e., Raf/MEK/ERK signaling), may be more attractive targets for reducing tumor growth in CaP patients.

MAPK Signaling and Drug Resistance Studies

The Raf/MEK/ERK signal transduction cascade is responsible for a number of cellular processes including proliferation, growth and differentiation. The relationship between Raf/MEK/ERK signaling and drug resistance is also well-documented. There are reports which propose that activation of the Raf/MEK/ERK pathway can increase the probability of survival in chemotoxic agents;[313,314] conversely, there is also an abundance of data which suggests that activation of ERK is a necessity for drug-induced apoptosis.[315-319] In prostatic disease, however, the role of ERK activation in chemoresistance remains controversial. At this time, there does not appear to be a single role for ERK in drug-induced cell death—instead, it seems that cell type and drug selection are two key factors in evaluating the role of ERK activation to drug treatments.

To examine the effects of ERK activity on the development of drug resistance in CaP cells, DU145 cells were stably-transfected with constitutively-activated mutants of both Raf-1 (ΔRaf-1) and BRaf (B-Raf G468A and B-Raf V600E).[320] In doing so, it was possible to ascertain the role(s) of Raf/MEK/ERK activation in altering the response of CaP cells to doxorubicin or paclitaxel treatment. Each of the constitutively-active mutants was able to increase activity of ERK, as measured by a phospho-specific antibody. Thus, it was then possible to move forward with these studies with the knowledge that the cell lines had increased activity of the Raf/MEK/ERK pathway when compared to non-transduced DU145 cells.

The effects of activation of the Raf kinase, via stable transfection of various DNA constructs, were then determined. The data,[320] were somewhat surprising because they showed that activation of the Raf/MEK/ERK pathway did not enhance the chemoresistance profile of DU145 cells. On the contrary, subsequent experiments have indicated that activation of this cascade upon restoration of WT p53, increased the sensitivity of the cells to both radiotherapy and chemotherapy.[250]

To confirm/refute the intriguing results, further experiments were performed. Those data demonstrate that inhibition of MAPK activity does little to enhance the killing capacity of doxorubicin and/or paclitaxel in DU145 cells. Together, these data confirm the data from previous studies and strongly suggest that activation of the Raf/MEK/ERK signaling cascade does not increase drug resistance in CaP cells. In fact activation of Raf/MEK/ERK may actually chemosensitize prostate cancer cells.

Initially, these results were somewhat surprising because our and other laboratories have previously reported[321-323] that activation of the Raf/MEK/ERK cascade provided protection from doxorubicin in the MCF-7 breast carcinoma and other cell lines. However, after a thorough literature search, it appears that activation of the Raf/MEK/ERK pathway has a pro-apoptotic effect when cancer cells are treated with chemotherapeutic agents in the majority of cell types. Interestingly, the pro-apoptotic action of drug-induced ERK activity is reported to be p53-dependent.[324,325] DU145 cells, however, possess a mutated p53 protein product which is insufficient in many of the activities associated with WT p53 activity (i.e., DNA damage-induced cell cycle arrest and p21 regulation, among others).[326] Therefore, it is quite feasible that activation of the Raf/MEK/ERK pathway is unable to induce chemoresistance in DU145 cells because of the absence of WT p53 function in this cell type. When a functional p53 gene was introduced into these cells, their chemo- and radiosensitivity increased dramatically as did the activation of the Raf/MEK/ERK cascade.

Intracellular signaling does not occur in a linear fashion. Instead, signaling between adjacent pathways is known to converge, cross and intersect one another in a complex, interwoven pattern that is generally undefined. Oftentimes, reports on crosstalk between pathways conflict each other, indicating that cell type must be considered when determining potential interactions within the cell. There are very few reports that have investigated the degree of crosstalk that occurs in cells of prostatic origin. Given the importance of the topic of these studies, it was vital to determine if potential crosstalk between the PI3K/PTEN/Akt and Raf/MEK/ERK pathways occurs and, if so, to what degree it affects drug resistance in CaP cells.

Dr. Jeffrey Kreisberg's laboratory (University of Texas Health Science Center, San Antonio) has demonstrated the importance of monitoring phospho-Akt levels as a prognostic tool for CaP patients.[327,328] In addition, they also report that decreased phospho-ERK levels are indicative of a poor prognosis.[327] Taken as a collective whole, the correlative data might suggest that activation of Akt is linked to the inactivation of ERK. This hypothesis was investigated further because preliminary data indicated that it may indeed be true.

It was soon discovered that inhibition of PI3K with LY294002 could cause increased levels of phospho-ERK.[329] This finding was subsequently verified using another inhibitor of PI3K, wortmannin. These results suggested that activated PI3K could mediate downregulation of the Raf/MEK/ERK pathway when active; on the contrary, inhibition of PI3K appears to activate the MAPK pathway.[329]

The complex phosphoregulation of Raf was described earlier; it is generally accepted that phosphorylation of S259 on Raf-1 is an event that inactivates the molecule from signaling downstream (to MEK).[330] To assess Raf activity in CaP cells, both DU145 and PC3 cells were assayed for their phospho-Raf-1^{S259} content. Interestingly, PC3 cells displayed much higher

levels of inactivated Raf-1 than the DU145 cell line.[329] This observation supports the hypothesis that activated PI3K/Akt can suppress the Raf/MEK/ERK pathway in a manner that involves Raf-1 inactivation.

To further test the hypothesis that the PI3K/Akt pathway causes inactivation of ERK through phosphorylation of Raf-1, reciprocal co-immunoprecipitation assays demonstrated that Akt and Raf-1 were found in conjunction with one another in PC3 cells; however, this was not as apparent in the DU145 cell type.[329] DU145 and PC3 cells were also subjected to low concentrations of doxorubicin and/or paclitaxel over two months and resistant cells were selected. DU145 cells selected in drug displayed elevated high levels of phospho-ERK, but relative levels of phospho-Akt when compared to wild-type cells.[329] PC3 cells, however, were unchanged in terms of phospho-Akt (high levels) and phospho-ERK (low). Together, these analyses support the notion that the absence of PTEN plays a major role in the inactivation of Raf-1 in PC3 cells, but not DU145 cells. While the evidence presented to prove that activation of ERK to potentiate the anti-apoptotic effects of doxorubicin and paclitaxel is minimal, the literature certainly supports this premise.[318,319,330-333] Consequently, it appears that PI3K activation may have an additional mechanism of action to confer drug resistance to CaP cells —inactivation of the Raf/MEK/ERK kinase cascade.

The significance of the studies performed is several-fold and has implications for not only acquired chemoresistance, but also normal progression of prostatic disease. The loss of *PTEN* expression appears to be a major factor in a series of events that can lead to drug-resistance and HRPC. After *PTEN*-loss and consequent activation of PI3K, resistance to chemotherapeutic compounds can occur in a number of ways. First, activation of PI3K was shown to upregulate expression of the *MRP-1* drug efflux pump gene through an unknown mechanism. Secondly, PI3K can activate Akt which can deactivate Raf by phosphorylation on S259; inactivation of Raf shuts down Raf/MEK/ERK signaling, which has been shown to be essential for chemotoxic drug-induced death. Although there exist alternative mechanisms which the CaP cell employs to become resistant to antineoplastic agents, steps can be taken now to exploit this new knowledge so that better therapies are present in the future.

Activation of Raf-1 and/or B-Raf was not capable of increasing the cellular resistance to either doxorubicin and/or paclitaxel. Initially, this was a troublesome finding because most believe that Raf/MEK/ERK activation leads to an intracellular state that enables the cell to more effectively respond to chemotoxic compounds. However, upon further analysis, it was discovered that this mechanism of action is dependent on the activity of the tumor suppressor protein, p53. Pertinent to these studies is that DU145 cells are p53 mutants (PC3: p53-null). Taken together, this data helps explain why activation of Raf-1 and B-Raf is insufficient to sensitize DU145 cells to doxorubicin and/or paclitaxel. Therefore, chemotherapeutic resistance proves to be a complex process depending on activates of multiple signaling pathways.

Radiosesitization of Prostate Cancer

Conventional-dose (72 Gy) external beam radiation is elected as a preferred treatment modality by many CaP patients.[334,335] However, there is evidence that conventional-dose radiation often does not provide complete tumor eradication with a resultant radiorecur-rent prostate cancer and five year distant metastasis-free survival of less than 80%.[336-338] Given this problem, it is not surprising that radiotherapy dose escalation has been recommended for patients with poor prognostic features (e.g., positive surgical margins);[339] but the toxicity associated with dose-escalated therapy is not trivial and it is recognized that 5-10% of all radiotherapy patients develop severe acute or late effects.[247] Accordingly, there is intense interest in understanding critical determinants in the efficacy of irradiation (IR)-induced CaP cell killing and developing targeted therapeutic strategies for radiosensitization.

Curative radiation therapy aims at preventing tumor regrowth by inducing tumor cell death and loss of reproductive integrity. These processes together are referred to as clonogenic death. Clonogenic cells are defined as tumor cells possessing the capacity to produce a colony of progeny and therefore contribute to the regrowth of the tumor. These cells are also more commonly referred to in other types of cancer as tumor stem cells or cancer-initiating cells.[340-346]

Radiation therapy creates substantial DNA damage that is recognized by p53 and forces most cells to either undergo programmed cell death (e.g., apoptosis), necrosis or enter a state of reproductive death (i.e., cellular senescence). Radiation therapy is conventionally delivered in fractions of 1.8-2.0 Gy doses for 5 days a week until a total of 60-80 Gy is reached. After sequential doses, more than 99% of the malignant cells are typically killed. However, the surviving fraction may amount to more than a million cells per gram of tumor volume.[347] These survivors retain the potential to actively contribute to radiorecurrent prostate cancer and are a major target for radiosensitization. However, the cellular and molecular heterogeneity of CaP make this subpopulation of radioresistant cells a difficult target to strike using a single modality of adjunctive therapy.[349]

Priming the p53 Pathway for Prostate Cancer Therapy

p53 activity is regulated by the E3 ubiquitin ligase MDM2, which binds and targets p53 for degradation.[350] Overexpression of MDM2 has been reported in prostate cancer cells and may serve to protect those cells from p53-mediated cellular senescence in response to therapy.[350] Thus, targeting MDM2 seems to be a reasonable target for sensitization to both chemotherapy and radiotherapy. Investigators have downregulated MDM2 using antisense and successfully sensitized LNCaP cells.[351,352] While antisense raised against MDM2 demonstrated that regulation of p53 is critical for sensitization, the use of antisense may prove to be difficult in the clinical setting.

Recently, a selective small-molecule inhibitor of MDM2-p53 binding, Nutlin-3 has been identified and characterized. Nutlin-3 disrupts MDM2 binding and p53 in cell culture at a concentration of 5-10 μM or inhibits tumor growth when given orally at 200 mg/kg.[353] Nutlin-3 specifically activated the p53 pathway in numerous cancer cells possessing WT p53, inducing cell cycle arrest and apoptosis to varying extents.[354] Since Nutlin-3 uncouples p53 from MDM2-mediated degradation, treatment with the drug prior to therapy may increase steady-state levels of p53, effectively priming the cellular ability to respond maximally to radiation. Investigators first demonstrated that administration of Nutlin-3 prior to therapy can effectively sensitize WT p53 lung cancer cells.[245] We have recently shown that disruption of p53-MDM2 interactions by the small-molecule MDM2 antagonist Nutlin-3 can effectively sensitize prostate cancer cells to a clinically-relevant dose (2 Gy) of IR, provided these CaP cells express WT p53.[250] Moreover, the increase in clonogenic cell death was entirely attributable to an increased induction of p53-dependent cellular senescence.

While Nutlin-3 may radiosensitize some tumors by “priming” the p53 pathway, it seems clear that this strategy will not apply to all prostate tumors. p53 activation requires both MDM2 degradation and MDMX inactivation. Nutlin-3 does not disrupt MDMX binding of MDM2 and MDMX overexpression prevents p53 stabilization by Nutlin-3.[354] Nutlin-3 disrupts MDM2 binding and inactivation of p53 in cell culture models. The observations suggest that further development of small-molecule inhibitors of MDMX, or alternative molecular targets, may be useful in combination with Nutlin-3 to provide a clinically effective radioresponse. Moreover, p53 activators could have an adverse outcome in patients with p53 GOF mutant alleles and it will be essential to determine their p53 status prior to initiating treatment.[355] Because prostate cancer is slow growing and is now often detected while still localized, the

clinician may some day have the opportunity to individualize treatment based on knowledge of the patient's p53 status.

AKT as an Adjunctive Target to p53 in Sensitization of Prostate Cancer

Small molecule inhibitors of PI3K such as LY294002 have been reported to effectively sensitize LNCaP and other prostate cancer cells.[281,356] Recently the novel Akt inhibitor, Perifosine, has been shown to have some effects on prostate cancer growth.[357] Thus, inactivating mutations of PTEN may limit therapy induced cell killing in prostate tumors due to unrestrained Akt activity. This may be related to the observation that the PI3K-Akt pathway effectively inhibits p53 by increasing its degradation via the MDM2 pathway.[358] More recently, however, it has been reported that activation of PI3K signaling results in the downstream activation of p53 and the p53-dependent senescence pathway.[359-361] This discrepancy may be the result of oncogenic stress stimulating p14, which would inactivate MDM2 regardless of the increased shuttling into the nucleus via Akt phosphorylation. Thus it is unclear whether targeting the Akt pathway in conjunction with p53 activation will synergize or antagonize the radioresponsiveness of prostate cancer cells. Future studies will be directed at resolving this controversy in order to understand how and when these tumor suppressor pathways interact to influence the radiosensitivity of CaP cells.

Conclusions

The developments of complimentary or alternative treatment strategies to standard chemo- and radiotherapies are invaluable to increase survival in patients with metastatic CaP. After hormone relapse, nearly 100% of patients succumb to this disease within 18 months. During this period, chemotherapy is administered primarily for palliation of symptoms associated with disease. Given the success of chemotherapeutic intervention in other cancer types, it stands to reason that it can be an effective means of treatment for prostatic disease as well. However, defining the mechanisms of chemoresistance within the prostatic tumor will be the caveat that enables this notion to become a reality.

Studies that investigate the mechanistic behavior of androgen-independent CaP cells are of the utmost importance in discovering better treatments with higher levels of efficacy. The genotype of the tumor must be considered when developing treatment regimens for a CaP patient as multiple signaling pathways feed into each other. Fortunately, it appears that clinical oncologists are headed in this direction,[361,362] as many physicians are beginning to push for the development of diagnostic tools that can be utilized to determine specific mutations that are present in a particular person's tumor. Taking into account the research discussed in this review, it appears that genotyping tumors will be an effective means of developing better treatment strategies for patients with HRPC, as the absence of a single gene (i.e., *PTEN*) can make a substantial difference in the response of patients to chemotherapeutic agents. In Figure 4, an overview is presented of the sites of mutation which have been identified in prostate cancer which result in activation of the PI3K/PTEN/Akt/mTOR and Raf/MEK/ERK pathways.

In previous publications, we have reported that the PI3K/PTEN/Akt/mTOR and Raf/MEK/ERK signaling pathways have synergistic effects which lead to the transformation of hematopoietic cells.[363-369] Recently, we reported that Akt activation can lead to inactivation of the MAPK pathway via Raf-1.[329] The results of these studies have importance in many arenas of prostatic research: disease progression and etiology, therapeutic efficacy and resistance to chemotoxic compounds.

PTEN, the negative regulator of PI3K signaling, is lost in a large percentage of prostatic carcinomas. Consequently, many patients with CaP harbor tumors with high levels of Akt signaling.[370,371] Our previous work demonstrated that aberrant Akt signaling can lead to

heightened levels of MRP-1 expression thereby causing increased resistance to doxorubicin and paclitaxel, two drugs commonly used to treat CaP patients.[281] Furthermore, we also demonstrated the role for Akt in the progression of CaP, namely inactivation of the Raf/MEK/ERK cascade.[329]

Signals transduced by the Raf/MEK/ERK cascade are generally thought to induce cellular growth, differentiation and/or proliferation.[372] Given that chemotoxic drugs target cells which divide rapidly, one would then hypothesize that cells with high levels of ERK activity would be more effectively targeted by these compounds. Conversely, the PI3K/Akt pathway is known for its role in cell survival—as such, cells with heightened Akt activity may not be as affected by treatment with chemotherapeutic drugs. Our previous reports[281,363] support this hypothesis.

Inactivation of ERK has been reported to coincide with higher Gleason grades and poorly differentiated CaP.[373] Due to its role in inducing differentiation, one hypothesis is that, in normal prostatic epithelial cells, ERK acts to induce cellular differentiation; however upon tumorigenesis, ERK is inactivated causing cells to differentiate much less efficiently, which is a hallmark of advanced CaP.[371] The data presented in our recent report support the notion that ERK is inactivated in advanced CaP.[329] Furthermore, evidence in this report[329] indicates that loss of PTEN and subsequent Akt activation is responsible for ERK inactivation via phosphorylation of Raf-1 on S259.[329] Lastly, others have shown that ERK activation is necessary for drug-induced death;[374-376] the findings herein suggest that administration of chemotoxic drugs may have minor deleterious effects in cells with heightened Akt activity (i.e., those with PTEN deletions), as they will serve to downregulate signaling within the Raf/MEK/ERK pathway. Tumor suppressors such as p53, PTEN and Rb play critical roles in prostate cancer growth. Clearly mutation at these tumor suppressors have multiple effects on cellular growth and death pathways. A summary of the effects of PTEN and p53 inactivation on PI3K/PTEN/Akt/mTOR and Raf/MEK/ERK in prostate cancer is presented in Figure 5.

Resistance to chemotherapeutic compounds is a problem that continues to impede the success of modern drug regimens.[377,378] Circumventing this resistance is an area of intense investigation due to the short life-span of patients who are afflicted with HRPC. Previously, we and others have identified the PI3K/Akt pathway as a primary mediator of drug resistance in advanced CaP.[281,379] After loss or mutation of PTEN, Akt becomes constitutively active thereby causing phosphorylation and inactivation of Raf-1 on S259.[329] Since this event is one that inactivates Raf-1, signals ordinarily transduced by the Raf/MEK/ERK cascade are negated. Consequently, the cell loses its ability to further differentiate, while heightened Akt activity confers a survival advantage to the cell. Together, these events may lead to a tumor microenvironment where the individual cells are not only resistant to chemotherapeutic compounds, but also fail to differentiate efficiently. While chemotherapeutics have had minimal success so far, the future for effective treatment may involve combinations with drugs simultaneously targeting signaling pathways (Akt, MAPK, p53). Clearly combining targeted therapy with chemotherapy as well as other therapeutic approaches may have significant potential in prostate cancer therapy as prostate cancer is a disease based in part on mutations in critical signaling and cell cycle regulatory genes which aberrantly regulate prostate cell growth.

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Abbreviations

AP-1, activator protein-1
ATM, ataxia teleangetasia mutated
ATR, ATM-related
CaP, prostate carcinoma
CDK, cyclin-dependent kinase
CREB, cyclic-AMP response element binding protein
DDR-1, discoidin domain receptor-1
DN, dominant negative
DSB, double strand break
FAK, focal adhesion kinase
FKHR (FOXO1), member of the forkhead/FoxO
4E-BP1-eIF, 4E binding protein-1
GAP, GTPase-activating protein
GOF, gain of function
GSK-3β, glycogen-synthase kinase-3β
ILK, integrin-linked kinase
IR, ionizing radiation
KSR, kinase suppressor of Ras
LOF, loss of function
MDM2, murine double-minute 2
MP1, MEK partner-1
mTOR, mammalian target of rapamycin
$p90^{RSK}$, p90 ribosomal S6 kinase
PDGF, platelet-derived growth factor
PDK, phosphotidylinositide-dependent kinases
PH, pleckstrin homology
PI3K, phosphosinotol-3 kinase
PKB, protein kinase B
PKC, protein kinase C
PP2A, phosphatase 2A
PIN, prostatic intraepithelial neoplasia
PTEN, phosphatase and tensin homologue deleted on chromosome ten
Rictor, mTOR complex
RKIP, Raf kinase inhibitor protein
SH2, Src-homology 2
SOS, son of sevenless
WT, wild type

References

1. Abate-Shen C, Shen MM. Molecular genetics of prostate cancer. Genes Dev 2000;14:2410–34. [PubMed: 11018010]
2. Morris DS, Tomlins SA, Rhodes DR, Mehra R, Shah RB, Chinnaiyah AM. Integrating biomedical knowledge to model pathways of prostate cancer progression. Cell Cycle 2007;6:1177–87. [PubMed: 17495538]
3. Merlet J, Moreau E, Habert R, Racine C. Development of fetal testicular cells in androgen receptor deficient mice. Cell Cycle 2007;6:2258–62. [PubMed: 17890904]
4. Kim MJ, Bhatia-Gaur R, Banach-Petrosky WA, Desai N, Wang Y, Hayward SW, Cunha GR, Cardiff RD, Shen MM, Abate-Shen C. Nkx3.1 mutant mice recapitulate early stages of prostate carcinogenesis. Cancer Res 2002;62:2999–3004. [PubMed: 12036903]

5. Bhatia-Gaur R, Donjacour AA, Sciavolino PJ, Kim M, Desai N, Young P, Norton CR, Gridley T, Cardiff RD, Cunha GR, Abate-Shen C, Shen MM. Roles for Nkx3.1 in prostate development and cancer. Genes Dev 1999;13:966–77. [PubMed: 10215624]
6. Bowen C, Bubendorf L, Voeller HJ, Slack R, Willi N, Sauter G, Gasser TC, Koivisto P, Lack EE, Kononen J, Kallioniemi OP, Gelmann EP. Loss of NKX3.1 expression in human prostate cancers correlates with tumor progression. Cancer Res 2000;60:6111–5. [PubMed: 11085535]
7. Vlietstra RJ, van Alewijk DC, Hermans KG, van Steenbrugge GJ, Trapman J. Frequent inactivation of PTEN in prostate cancer cell lines and xenografts. Cancer Res 1998;58:2720–3. [PubMed: 9661880]
8. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast and prostate cancer. Science 1997;275:1943–7. [PubMed: 9072974]
9. Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DH, Tavtigian SV. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 1997;15:356–62. [PubMed: 9090379]
10. Jonason JH, Gavrilova N, Wu M, Zhang H, Sun H. Regulation of SCF(SKP2) ubiquitin E3 ligase assembly and p27(KIP1) proteolysis by the PTEN pathway and cyclin D1. Cell Cycle 2007;6:951–61. [PubMed: 17438373]
11. Tsang CK, Zheng XF. TOR-in(g) the nucleus. Cell Cycle 2007;6:25–9. [PubMed: 17245124]
12. Teng DH, Hu R, Lin H, Davis T, Iliev D, Frye C, Swedlund B, Hansen KL, Vinson VL, Gumpper KL, Ellis L, El-Naggar A, Frazier M, Jasser S, Langford LA, Lee J, Mills GB, Pershouse MA, Pollack RE, Tornos C, Troncoso P, Yung WK, Fujii G, Berson A, Bookstein R, Bolen JB, Tabtigian SV, Steck PA. MMAC1/PTEN mutations in primary tumor specimens and tumor cell lines. Cancer Res 1997;57:5221–5. [PubMed: 9393738]
13. Bookstein R, Shew JY, Chen PL, Scully P, Lee WH. Suppression of tumorigenicity of human prostate carcinoma cells by replacing a mutated RB gene. Science 1990;247:712–5. [PubMed: 2300823]
14. Melamed J, Einhorn JM, Ittmann MM. Allelic loss on chromosome 13q in human prostate carcinoma. Clin Cancer Res 1997;3:1867–72. [PubMed: 9815575]
15. Cooney KA, Wetzel JC, Merajver SD, Macoska JA, Singleton TP, Wojno KJ. Distinct regions of allelic loss on 13q in prostate cancer. Cancer Res 1996;56:1142–5. [PubMed: 8640774]
16. Bosco EE, Knudsen ES. Rb in breast cancer: at the crossroads of tumorigenesis and treatment. Cell Cycle 2007;6:667–71. [PubMed: 17361100]
17. Chang F, Lee JT, Navolanic PM, Steelman LS, Shelton JG, Blalock WL, Franklin RA, McCubrey JA. Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis and neoplastic transformation: a target for cancer chemotherapy. Leukemia 2003;17:590–603. [PubMed: 12646949]
18. Toker A, Cantley LC. Signalling through the lipid products of phosphoinositide-3-OH kinase. Nature 1997;387:673–6. [PubMed: 9192891]
19. Staal SP, Hartley JW. Thymic lymphoma induction by the AKT8 murine retrovirus. J Exp Med 1988;167:1259–64. [PubMed: 2832508]
20. Staal SP. Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. Proc Natl Acad Sci USA 1987;84:5034–7. [PubMed: 3037531]
21. Franke TF, Yang SI, Chan TO, Datta K, Kazlauskas A, Morrison DK, Kaplan DR, Tsichlis PN. The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. Cell 1995;81:727–36. [PubMed: 7774014]
22. Franke TF, Kaplan DR, Cantley LC, Toker A. Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. Science 1997;275:665–8. [PubMed: 9005852]
23. Datta K, Franke TF, Chan TO, Makris A, Yang SI, Kaplan DR, Morrison DK, Golemis EA, Tsichlis PN. AH/PH domain-mediated interaction between Akt molecules and its potential role in Akt regulation. Mol Cell Biol 1995;15:2304–10. [PubMed: 7891724]
24. Coffer PJ, Woodgett JR. Molecular cloning and characterisation of a novel putative protein-serine kinase related to the cAMP-dependent and protein kinase C families. Eur J Biochem 1991;201:475–81. [PubMed: 1718748]

25. Bellacosa A, Testa JR, Staal SP, Tsichlis PN. A retroviral oncogene, akt, encoding a serinethreonine kinase containing an SH2-like region. Science 1991;254:274–7. [PubMed: 1833819]
26. Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA. Mechanism of activation of protein kinase B by insulin and IGF-1. Embo J 1996;15:6541–51. [PubMed: 8978681]
27. Scheid MP, Woodgett JR. Unravelling the activation mechanisms of protein kinase B/Akt. FEBS Lett 2003;546:108–12. [PubMed: 12829245]
28. Scheid MP, Marignani PA, Woodgett JR. Multiple phosphoinositide 3-kinase-dependent steps in activation of protein kinase B. Mol Cell Biol 2002;22:6247–60. [PubMed: 12167717]
29. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P. Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. Curr Biol 1997;7:261–9. [PubMed: 9094314]
30. Toker A, Newton AC. Akt/protein kinase B is regulated by autophosphorylation at the hypothetical PDK-2 site. J Biol Chem 2000;275:8271–4. [PubMed: 10722653]
31. Delcommenne M, Tan C, Gray V, Rue L, Woodgett J, Dedhar S. Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the integrin-linked kinase. Proc Natl Acad Sci USA 1998;95:11211–6. [PubMed: 9736715]
32. Lynch DK, Ellis CA, Edwards PA, Hiles ID. Integrin-linked kinase regulates phosphorylation of serine 473 of protein kinase B by an indirect mechanism. Oncogene 1999;18:8024–32. [PubMed: 10637513]
33. Steelman LS, Abrams SL, Whelan J, Bertrand FE, Ludwig DE, Bäsecke J, Libra M, Stivala F, Milella M, Tafuri A, Lunghi P, Bonati A, Martelli AM, McCubrey JA. Contributions of the Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways to leukemia. Leukemia. 2008In Press
34. Martelli AM, Tazzari PL, Evangelisti C, Chiarini F, Blalock WL, Billi AM, Manzoli L, McCubrey JA, Cocco L. Targeting the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin module for acute myelogenous leukemia therapy: from bench to bedside. Curr Med Chem 2007;14:2009–23. [PubMed: 17691943]
35. Andjelkovic M, Alessi DR, Meier R, Fernandez A, Lamb NJ, Frech M, Cron P, Cohen P, Lucocq JM, Hemmings BA. Role of translocation in the activation and function of protein kinase B. J Biol Chem 1997;272:31515–24. [PubMed: 9395488]
36. Du K, Montminy M. CREB is a regulatory target for the protein kinase Akt/PKB. J Biol Chem 1998;273:32377–9. [PubMed: 9829964]
37. Brennan P, Babbage JW, Burgering BM, Groner B, Reif K, Cantrell DA. Phosphatidylinositol 3-kinase couples the interleukin-2 receptor to the cell cycle regulator E2F. Immunity 1997;7:679–89. [PubMed: 9390691]
38. McClellan KA, Slack RS. Specific in vivo roles for E2Fs in differentiation and development. Cell Cycle 2007;6:2917–27. [PubMed: 17993781]
39. Assoian RK, Yung Y. A reciprocal relationship between Rb and Skp2: implications for restriction point control, signal transduction to the cell cycle and cancer. Cell Cycle 2008;7:24–7. [PubMed: 18196971]
40. Kane LP, Shapiro VS, Stokoe D, Weiss A. Induction of NFkappaB by the Akt/PKB kinase. Curr Biol 1999;9:601–4. [PubMed: 10359702]
41. Vigano MA, Mantovani R. Hitting the numbers: the emerging network of p63 targets. Cell Cycle 2007;6:233–9. [PubMed: 17297297]
42. Kops GJ, Burgering BM. Forkhead transcription factors are targets of signalling by the proto-oncogene PKB (C-AKT). J Anat 2000;197:571–4. [PubMed: 11197530]
43. Nakamura N, Ramaswamy S, Vazquez F, Signoretti S, Loda M, Sellers WR. Forkhead transcription factors are critical effectors of cell death and cell cycle arrest downstream of PTEN. Mol Cell Biol 2000;20:8969–82. [PubMed: 11073996]
44. Medema RH, Kops GJ, Bos JL, Burgering BM. AFX-like Forkhead transcription factors mediate cell cycle regulation by Ras and PKB through $p27^{kip1}$. Nature 2000;404:782–7. [PubMed: 10783894]
45. Biggs WH, Meisenhelder J 3rd, Hunter T, Cavenee WK, Arden KC. Protein kinase B/Akt-mediated phosphorylation promotes nuclear exclusion of the winged helix transcription factor FKHR1. Proc Natl Acad Sci USA 1999;96:7421–6. [PubMed: 10377430]

46. Rena G, Guo S, Cichy SC, Unterman TG, Cohen P. Phosphorylation of the transcription factor forkhead family member FKHR by protein kinase B. J Biol Chem 1999;274:17179–83. [PubMed: 10358075]

47. Tang ED, Nunez G, Barr FG, Guan KL. Negative regulation of the forkhead transcription factor FKHR by Akt. J Biol Chem 1999;274:16741–6. [PubMed: 10358014]

48. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 1999;96:857–68. [PubMed: 10102273]

49. Kops GJ, de Ruiter ND, De Vries-Smits AM, Powell DR, Bos JL, Burgering BM. Direct control of the Forkhead transcription factor AFX by protein kinase B. Nature 1999;398:630–4. [PubMed: 10217147]

50. Nakae J, Park BC, Accili D. Insulin stimulates phosphorylation of the forkhead transcription factor FKHR on serine 253 through a Wortmannin-sensitive pathway. J Biol Chem 1999;274:15982–5. [PubMed: 10347145]

51. Cardone MH, Roy N, Stennicke HR, Salvesen GS, Franke TF, Stanbridge E, Frisch S, Reed JC. Regulation of cell death protease caspase-9 by phosphorylation. Science 1998;282:1318–21. [PubMed: 9812896]

52. del Peso L, Gonzalez-Garcia M, Page C, Herrera R, Nunez G. Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. Science 1997;278:687–9. [PubMed: 9381178]

53. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 1997;91:231–41. [PubMed: 9346240]

54. Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 1995;378:785–9. [PubMed: 8524413]

55. Weng LP, Brown JL, Baker KM, Ostrowski MC, Eng C. PTEN blocks insulin-mediated ETS-2 phosphorylation through MAP kinase, independently of the phosphoinositide 3-kinase pathway. Hum Mol Genet 2002;11:1687–96. [PubMed: 12095911]

56. Kang-Park S, Lee YI. PTEN modulates insulin-like growth factor II (IGF-II)-mediated signaling; the protein phosphatase activity of PTEN downregulates IGF-II expression in hepatoma cells. FEBS Lett 2003;545:203–8. [PubMed: 12804776]

57. Mahimainathan L, Choudhury GG. Inactivation of platelet-derived growth factor receptor by the tumor suppressor PTEN provides a novel mechanism of action of the phosphatase. J Biol Chem 2004;279:15258–68. [PubMed: 14718524]

58. Myers MP, Pass I, Batty IH, Van der Kaay J, Stolarov JP, Hemmings BA, Wigler MH, Downes CP, Tonks NK. The lipid phosphatase activity of PTEN is critical for its tumor supressor function. Proc Natl Acad Sci USA 1998;95:13513–8. [PubMed: 9811831]

59. Maehama T, Dixon JE. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. J Biol Chem 1998;273:13375–8. [PubMed: 9593664]

60. Maier D, Jones G, Li X, Schontha AH, Gratzl O, Van Meir EG, Merlo A. The PTEN lipid phosphatase domain is not required to inhibit invasion of glioma cells. Cancer Res 1999;59:5479–82. [PubMed: 10554022]

61. Pesche S, Latil A, Muzeau F, Cussenot O, Fournier G, Longy M, Eng C, Lidereau R. PTEN/MMAC1/TEP1 involvement in primary prostate cancers. Oncogene 1998;16:2879–83. [PubMed: 9671408]

62. Ittmann MM. Chromosome 10 alterations in prostate adenocarcinoma (review). Oncol Rep 1998;5:1329–35. [PubMed: 9769364]

63. Kreisberg JI, Malik SN, Prihoda TJ, Bedolla RG, Troyer DA, Kreisberg S, Ghosh PM. Phosphorylation of Akt (Ser473) is an excellent predictor of poor clinical outcome in prostate cancer. Cancer Res 2004;64:5232–6. [PubMed: 15289328]

64. Ghosh PM, Malik S, Bedolla R, Kreisberg JI. Akt in prostate cancer: possible role in androgen-independence. Curr Drug Metab 2003;4:487–96. [PubMed: 14683476]

65. Guo Y, Sklar GN, Borkowski A, Kyprianou N. Loss of the cyclin-dependent kinase inhibitor p27 (Kip1) protein in human prostate cancer correlates with tumor grade. Clin Cancer Res 1997;3:2269–74. [PubMed: 9815624]

66. McAndrew CW, Gastwirt RF, Meyer AN, Porter LA, Donoghue DJ. Spy1 enhances phosphorylation and degradation of the cell cycle inhibitor p27. Cell Cycle 2007;6:1937–45. [PubMed: 17671428]

67. Borriello A, Cucciolla V, Oliva A, Zappia V, Della Ragione F. $p27^{Kip1}$ metabolism: a facinating labyrinth. Cell Cycle 2007;6:1053–61. [PubMed: 17426451]

68. Chien WM, Garrison K, Caufield E, Orthel J, Dill J, Fero ML. Differential gene expression of $p27^{Kip1}$ and Rb knockout pituitary tumorss associated with altered growth and angiogenesis. Cell Cycle 2007;6:750–7. [PubMed: 17361101]

69. Surjit M, Lal SK. Glycogen synthase kinase-3 phosphorylates and regulates the stability of $p27^{Kip1}$ protein. Cell Cycle 2007;6:580–8. [PubMed: 17351340]

70. Di Cristofano A, De Acetis M, Koff A, Cordon-Cardo C, Pandolfi PP. Pten and $p27^{KIP1}$ cooperate in prostate cancer tumor suppression in the mouse. Nat Genet 2001;27:222–4. [PubMed: 11175795]

71. Medema RH, Kops GJ, Bos JL, Burgering BM. AFX-like Forkhead transcription factors mediate cell cycle regulation by Ras and PKB through $p27^{kip1}$. Nature 2000;404:782–7. [PubMed: 10783894]

72. Dijkers PF, Medema RH, Pals C, Banerji L, Thomas NS, Lam EW, Burgering BM, Raaijmakers JA, Lammers JW, Koenderman L, Coffer PJ. Forkhead transcription factor FKHR-L1 modulates cytokine-dependent transcriptional regulation of p27(KIP1). Mol Cell Biol 2000;20:9138–48. [PubMed: 11094066]

73. Mamillapalli R, Gavrilova N, Mihaylova VT, Tsvetkov LM, Wu H, Zhang H, Sun H. PTEN regulates the ubiquitin-dependent degradation of the CDK inhibitor p27(KIP1) through the ubiquitin E3 ligase SCF(SKP2). Curr Biol 2001;11:263–7. [PubMed: 11250155]

74. Graff JR, Konicek BW, McNulty AM, Wang Z, Houck K, Allen S, Paul JD, Hbaiu A, Goode RG, Sandusky GE, Vessella RL, Neubauer BL. Increased AKT activity contributes to prostate cancer progression by dramatically accelerating prostate tumor growth and diminishing $p27^{Kip1}$ expression. J Biol Chem 2000;275:24500–5. [PubMed: 10827191]

75. Murillo H, Huang H, Schmidt LJ, Smith DI, Tindall DJ. Role of PI3K signaling in survival and progression of LNCaP prostate cancer cells to the androgen refractory state. Endocrinology 2001;142:4795–805. [PubMed: 11606446]

76. Datta K, Franke TF, Chan TO, Makris A, Yang SI, Kaplan DR, Morrison DK, Golemis EA, Tsichlis PN. AH/PH domain-mediated interaction between Akt molecules and its potential role in Akt regulation. Mol Cell Bio 1995;15:2304–10. [PubMed: 7891724]

77. Fletcher JI, Huang DC. Controlling the cell death mediators Bax and Bak: puzzles and conundrums. Cell Cycle 2008;7:39–44. [PubMed: 18196961]

78. Kennedy SG, Kandel ES, Cross TK, Hay N. Akt/Protein kinase B inhibits cell death by preventing the release of cytochrome c from mitochondria. Mol Cell Biol 1999;19:5800–10. [PubMed: 10409766]

79. Dijkers PF, Medema RH, Lammers JW, Koenderman L, Coffer PJ. Expression of the pro-apoptotic Bcl-2 family member Bim is regulated by the forkhead transcription factor FKHR-L1. Curr Biol 2000;10:1201–4. [PubMed: 11050388]

80. Gingras AC, Kennedy SG, O'Leary MA, Sonenberg N, Hay N. 4E-BP1, a repressor of mRNA translation, is phosphorylated and inactivated by the Akt(PKB) signaling pathway. Genes Dev 1998;12:502–13. [PubMed: 9472019]

81. Gingras AC, Raught B, Sonenberg N. mTOR signaling to translation. Curr Top Microbiol Immunol 2004;279:169–97. [PubMed: 14560958]

82. Raught B, Gingras AC, Sonenberg N. The target of rapamycin (TOR) proteins. Proc Natl Acad Sci USA 2001;98:7037–44. [PubMed: 11416184]

83. Aoki M, Blazek E, Vogt PK. A role of the kinase mTOR in cellular transformation induced by the oncoproteins P3k and Akt. Proc Natl Acad Sci USA 2001;98:136–41. [PubMed: 11134523]

84. Sekulic A, Hudson CC, Homme JL, Yin P, Otterness DM, Karnitz LM, Abraham RT. A direct linkage between the phosphoinositide 3-kinase-AKT signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells. Cancer Res 2000;60:3504–13. [PubMed: 10910062]

85. Huang J, Klionsky DJ. Autophagy and human disease. Cell Cycle 2007;6:1837–49. [PubMed: 17671424]

86. Moretti L, Cha YI, Niermann KJ, Lu B. Switch between apoptosis and autophagy: radiation-induced endoplasic reticulum stress? Cell Cycle 2007;6:793–8. [PubMed: 17377498]

87. Tasdemir E, Maiuri MC, Tajeddine N, Vitale I, Criollo A, Vicencio JM, Hickman JA, Geneste O, Kroemer G. Cell cycle-dependent induction of autophagy, mitophagy and reticulophagy. Cell Cycle 2007;6:2263–7. [PubMed: 17890908]

88. Graff JR, Zimmer SG. Translational control and metastatic progression: enhanced activity of the mRNA cap-binding protein eIF-4E selectively enhances translation of metastasis-related mRNAs. Clin Exp Metastasis 2003;20:265–73. [PubMed: 12741684]

89. Zimmer SG, DeBenedetti A, Graff JR. Translational control of malignancy: the mRNA cap-binding protein, eIF-4E, as a central regulator of tumor formation, growth, invasion and metastasis. Anticancer Res 2000;20:1343–51. [PubMed: 10928042]

90. Galmozzi E, Casalini P, Iorio MV, Casati B, Olgiati C, Menard S. HER2 signaling enhances 5'UTR-mediated translation of c-Myc mRNA. J Cell Physiol 2004;200:82–8. [PubMed: 15137060]

91. Britton S, Salles B, Calsou P. c-MYC protein is degraded in response to UV irraditation. Cell Cycle 2008;7:63–70. [PubMed: 18196973]

92. Cowling VH, Cole MD. Turning the tables: Myc activates Wnt in breast cancer. Cell Cycle 2007;6:2625–7. [PubMed: 17726380]

93. Bell E, Lunec J, Tweddle DA. Cell Cycle regulation targets of MYCN indentified by gene expression microarrays. Cell Cycle 2007;6:1249–56. [PubMed: 17495526]

94. Nieminen AI, Partanen JI, Klefstrom J. c-Myc blazing a trail of death: coupling of the mitochondrial and death receptor apoptosis pathways by c-Myc. Cell Cycle 2007;6:2464–72. [PubMed: 17914284]

95. Prochownik EV, Li Y. The ever expanding role for c-Myc in promoting genomic instability. Cell Cycle 2007;6:1024–9. [PubMed: 17426456]

96. Efstratiadis A, Szabolcs M, Klinakis A. Notch, Myc and breast cancer. Cell Cycle 2007;6:418–29. [PubMed: 17329972]

97. Gera JF, Mellinghoff IK, Shi Y, Rettig MB, Tran C, Hsu JH, Sawyers CL, Lichtenstein AK. AKT activity determines sensitivity to mammalian target of rapamycin (mTOR) inhibitors by regulating cyclin D1 and c-myc expression. J Biol Chem 2004;279:2737–46. [PubMed: 14576155]

98. Klein EA, Yang C, Kazanietz MG, Assoian RK. NFkappaB-independent signaling to the cyclin D1 gene by Rac. Cell Cycle 2007:1115–21. [PubMed: 17426454]

99. Chung J, Bachelder RE, Lipscomb EA, Shaw LM, Mercurio AM. Integrin (alpha 6 beta 4) regulation of eIF-4E activity and VEGF translation: a survival mechanism for carcinoma cells. J Cell Biol 2002;158:165–74. [PubMed: 12105188]

100. Drobnjak M, Osman I, Scher HI, Fazzari M, Cordon-Cardo C. Overexpression of cyclin D1 is associated with metastatic prostate cancer to bone. Clin Cancer Res 2000;6:1891–5. [PubMed: 10815912]

101. Han EK, Lim JT, Arber N, Rubin MA, Xing WQ, Weinstein IB. Cyclin D1 expression in human prostate carcinoma cell lines and primary tumors. Prostate 1998;35:95–101. [PubMed: 9568672]

102. Dunsmuir WD, Gillett CE, Meyer LC, Young MP, Corbishley C, Eeles RA, Kirby RS. Molecular markers for predicting prostate cancer stage and survival. BJU Int 2000;86:869–78. [PubMed: 11069416]

103. Mousses S, Wagner U, Chen Y, Kim JW, Bubendorf L, Bittner M, Pretlow T, Elkahloun AG, Trepel JB, Kallioniemi OP. Failure of hormone therapy in prostate cancer involves systematic restoration of androgen responsive genes and activation of rapamycin sensitive signaling. Oncogene 2001;20:6718–23. [PubMed: 11709706]

104. Majumder PK, Febbo PG, Bikoff R, Berger R, Xue Q, McMahon LM, Manola J, Brugarolas J, McDonnell TJ, Golub TR, Loda M, Lane HA, Sellers WR. mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. Nat Med 2004;10:594–601. [PubMed: 15156201]

105. Singh D, Febbo PG, Ross K, Jackson DG, Manola J, Ladd C, Tamayo P, Renshaw AA, D'Amico AV, Richie JP, Lander ES, Loda M, Kantoff PW, Golub TR, Sellers WR. Gene expression correlates of clinical prostate cancer behavior. Cancer Cell 2002;1:203–9. [PubMed: 12086878]

106. Gao N, Zhang Z, Jiang BH, Shi X. Role of PI3K/AKT/mTOR signaling in the cell cycle progression of human prostate cancer. Biochem Biophys Res Commun 2003;310:1124–32. [PubMed: 14559232]
107. Dengjel J, Akimov V, Blagoey B, Andersen JS. Signal transduction by grown factor receptors: signaling in an instant. Cell Cycle 2007;6:2913–6. [PubMed: 18000401]
108. Chang F, Steelman LS, Lee JT, Shelton JG, Navolanic PM, Blalock WL, Franklin RA, McCubrey JA. Signal transduction mediated by the Ras/Raf/MEK/ERK pathway from cytokine receptors to transcription factors: potential targeting for therapeutic intervention. Leukemia 2003;17:1263–93. [PubMed: 12835716]
109. Lee JT Jr, McCubrey JA. The Raf/MEK/ERK signal transduction cascade as a target for chemotherapeutic intervention in leukemia. Leukemia 2002;16:486–507. [PubMed: 11960326]
110. Motti ML, De Marco C, Califano D, De Gisi S, Malanga D, Troncone G, Persico A, Losito S, Fabiani F, Santoro M, Chiappetta G, Fusco A, Viglietto G. Loss of p27 expression through Ras→BRAF→MAP kinase-dependent pathway in human thyroid carcinomas. Cell Cycle 2007;6:2817–25. [PubMed: 18032931]
111. Le Sage C, Nagel R, Agami R. Diverse ways to control $p27^{Kip1}$ function: miRNAs come into play. Cell Cycle 2007;6:2742–9. [PubMed: 17986865]
112. Ramjaun AR, Downward J. Ras and phosphoinositide 3-kinase: partners in development and tumorigensis. Cell Cycle 2007;6:2902–5. [PubMed: 17993782]
113. Lee K, Song K. Actin dysfunction activates ERK1/2 and delays entry into mitosis in mammalian cells. Cell Cycle 2007;6:1487–95. [PubMed: 17582224]
114. Pimienta G, Pascual J. Canonical and alternative MAPK signaling. Cell Cycle 2007;6:2628–32. [PubMed: 17957138]
115. Birge RB, Knudsen BS, Besser D, Hanafusa H. SH2 and SH3-containing adaptor proteins: redundant or independent mediators of intracellular signal transduction. Genes Cells 1996;1:595–613. [PubMed: 9078388]
116. Peehl DM. Oncogenes in prostate cancer. An update. Cancer 1993;71:1159–64.
117. Gumerlock PH, Poonamallee UR, Meyers FJ, deVere White RW. Activated ras alleles in human carcinoma of the prostate are rare. Cancer Res 1991;51:1632–7. [PubMed: 1998954]
118. Moul JW, Friedrichs PA, Lance RS, Theune SM, Chang EH. Infrequent RAS oncogene mutations in human prostate cancer. Prostate 1992;20:327–38. [PubMed: 1608859]
119. Buday L, Downward J. Epidermal growth factor regulates $p21^{ras}$ through the formation of a complex of receptor, Grb2 adapter protein, and Sos nucleotide exchange factor. Cell 1993;73:611–20. [PubMed: 8490966]
120. Moodie SA, Willumsen BM, Weber MJ, Wolfman A. Complexes of Ras. GTP with Raf-1 and mitogen-activated protein kinase kinase. Science 1993;260:1658–61. [PubMed: 8503013]
121. Vojtek AB, Hollenberg SM, Cooper JA. Mammalian Ras interacts directly with the serine/threonine kinase Raf. Cell 1993;74:205–14. [PubMed: 8334704]
122. Zhang XF, Settleman J, Kyriakis JM, Takeuchi-Suzuki E, Elledge SJ, Marshall MS, Bruder JT, Rapp UR, Avruch J. Normal and oncogenic $p21^{ras}$ proteins bind to the amino-terminal regulatory domain of c-Raf-1. Nature 1993;364:308–13. [PubMed: 8332187]
123. Warne PH, Viciana PR, Downward J. Direct interaction of Ras and the amino-terminal region of Raf-1 in vitro. Nature 1993;364:352–5. [PubMed: 8332195]
124. Ross EM, Wilkie TM. GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. Annu Rev Biochem 2000;69:795–827. [PubMed: 10966476]
125. Kolch W, Kotwaliwale A, Vass K, Janosch P. The role of Raf kinases in malignant transformation. Expert Rev Mol Med 2002;4:1–18. [PubMed: 14987392]
126. O'Neill E, Kolch W. Conferring specificity on the ubiquitous Raf/MEK signalling pathway. Br J Cancer 2004;90:283–8. [PubMed: 14735164]
127. Kolch W. Ras/Raf signalling and emerging pharmacotherapeutic targets. Expert Opin Pharmacother 2002;3:709–18. [PubMed: 12036410]

128. Hagemann C, Rapp UR. Isotype-specific functions of Raf kinases. Exp Cell Res 1999;253:34–46. [PubMed: 10579909]
129. Van Aelst L, Barr M, Marcus S, Polverino A, Wigler M. Complex formation between RAS and RAF and other protein kinases. Proc Natl Acad Sci USA 1993;90:6213–7. [PubMed: 8327501]
130. Erpel T, Courtneidge SA. Src family protein tyrosine kinases and cellular signal transduction pathways. Curr Opin Cell Biol 1995;7:176–82. [PubMed: 7612268]
131. Sozeri O, Vollmer K, Liyanage M, Frith D, Kour G, Mark GE 3rd, Stabel S. Activation of the c-Raf protein kinase by protein kinase C phosphorylation. Oncogene 1992;7:2259–62. [PubMed: 1437148]
132. Kolch W, Heidecker G, Kochs G, Hummel R, Vahidi H, Mischak H, Finkenzeller G, Marme D, Rapp UR. Protein kinase C alpha activates RAF-1 by direct phosphorylation. Nature 1993;364:249–52. [PubMed: 8321321]
133. Dhillon AS, Kolch W. Untying the regulation of the Raf-1 kinase. Arch Biochem Biophys 2002;404:3–9. [PubMed: 12127063]
134. Dhillon AS, Meikle S, Yazici Z, Eulitz M, Kolch W. Regulation of Raf-1 activation and signalling by dephosphorylation. Embo J 2002;21:64–71. [PubMed: 11782426]
135. Li S, Janosch P, Tanji M, Rosenfeld GC, Waymire JC, Mischak H, Kolch W, Sedivy JM. Regulation of Raf-1 kinase activity by the 14-3-3 family of proteins. Embo J 1995;14:685–96. [PubMed: 7882972]
136. Heidecker G, Kolch W, Morrison DK, Rapp UR. The role of Raf-1 phosphorylation in signal transduction. Adv Cancer Res 1992;58:53–73. [PubMed: 1312290]
137. Tzivion G, Luo Z, Avruch JA. Dimeric 14-3-3 protein is an essential cofactor for Raf kinase activity. Nature 1998;394:88–92. [PubMed: 9665134]
138. Avruch J, Khokhlatchev A, Kyriakis JM, Luo Z, Tzivion G, Vavvas D, Zhang XF. Ras activation of the Raf kinase: tyrosine kinase recruitment of the MAP kinase cascade. Recent Prog Horm Res 2001;56:127–55. [PubMed: 11237210]
139. Kubicek M, Pacher M, Abraham D, Podar K, Eulitz M, Baccarini M. Dephosphorylation of Ser-259 regulates Raf-1 membrane association. J Biol Chem 2002;277:7913–9. [PubMed: 11756411]
140. Abraham D, Podar K, Pacher M, Kubicek M, Welzel N, Hemmings BA, Dilworth SM, Mischak H, Kolch W, Baccarini M. Raf-1-associated protein phosphatase 2A as a positive regulator of kinase activation. J Biol Chem 2000;275:22300–4. [PubMed: 10801873]
141. Dhillon AS, Meikle S, Peyssonnaux C, Grindlay J, Kaiser C, Steen H, Shaw PE, Mischak H, Eychene A, Kolch W. A Raf-1 mutant that dissociates MEK/extracellular signal-regulated kinase activation from malignant transformation and differentiation but not proliferation. Mol Cell Biol 2003;23:1983–93. [PubMed: 12612072]
142. Mason CS, Springer CJ, Cooper RG, Superti-Furga G, Marshall CJ, Marais R. Serine and tyrosine phosphorylations cooperate in Raf-1, but not B-Raf activation. Embo J 1999;18:2137–48. [PubMed: 10205168]
143. Chiloeches A, Mason CS, Marais R. S338 phosphorylation of Raf-1 is independent of phosphatidylinositol 3-kinase and Pak3. Mol Cell Biol 2001;21:2423–34. [PubMed: 11259591]
144. Zheng CF, Guan KL. Activation of MEK family kinases requires phosphorylation of two conserved Ser/Thr residues. Embo J 1994;13:1123–31. [PubMed: 8131746]
145. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev 2001;22:153–83. [PubMed: 11294822]
146. Morrison DK. KSR: a MAPK scaffold of the Ras pathway? J Cell Sci 2001;114:1609–12. [PubMed: 11309192]
147. Schaeffer HJ, Catling AD, Eblen ST, Collier LS, Krauss A, Weber MJ. MP1: a MEK binding partner that enhances enzymatic activation of the MAP kinase cascade. Science 1998;281:1668–71. [PubMed: 9733512]
148. Keller ET, Fu Z, Brennan M. The biology of a prostate cancer metastasis suppressor protein: Raf kinase inhibitor protein. J Cell Biochem 2005;94:273–8. [PubMed: 15565643]

149. Fu Z, Kitagawa Y, Shen R, Shah R, Mehra R, Rhodes D, Keller PJ, Mizokami A, Dunn R, Chinnaiyan AM, Yao Z, Keller ET. Metastasis suppressor gene Raf kinase inhibitor protein (RKIP) is a novel prognostic marker in prostate cancer. Prostate 2006;66:248–56. [PubMed: 16175585]

150. Yeung K, Seitz T, Li S, Janosch P, McFerran B, Kaiser C, Fee F, Katsanakis KD, Rose DW, Mischak H, Sedivy JM, Kolch W. Suppression of Raf-1 kinase activity and MAP kinase signalling by RKIP. Nature 1999;401:173–7. [PubMed: 10490027]

151. Zheng CF, Guan KL. Properties of MEKs, the kinases that phosphorylate and activate the extracellular signal-regulated kinases. J Biol Chem 1993;268:23933–9. [PubMed: 8226933]

152. Crews CM, Alessandrini A, Erikson RL. The primary structure of MEK, a protein kinase that phosphorylates the ERK gene product. Science 1992;258:478–80. [PubMed: 1411546]

153. Khokhlatchev AV, Canagarajah B, Wilsbacher J, Robinson M, Atkinson M, Goldsmith E, Cobb MH. Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. Cell 1998;93:605–15. [PubMed: 9604935]

154. Shapiro PS, Whalen AM, Tolwinski NS, Wilsbacher J, Froelich-Ammon SJ, Garcia M, Osheroff N, Ahn NG. Extracellular signal-regulated kinase activates topoisomerase IIalpha through a mechanism independent of phosphorylation. Mol Cell Biol 1999;19:3551–60. [PubMed: 10207078]

155. Sturgill TW, Ray LB, Erikson E, Maller JL. Insulin-stimulated MAP-2 kinase phosphorylates and activates ribosomal protein S6 kinase II. Nature 1988;334:715–8. [PubMed: 2842685]

156. Xing J, Ginty DD, Greenberg ME. Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. Science 1996;273:959–63. [PubMed: 8688081]

157. Joel PB, Smith J, Sturgill TW, Fisher TL, Blenis J, Lannigan DA. pp90rsk1 regulates estrogen receptor-mediated transcription through phosphorylation of Ser-167. Mol Cell Biol 1998;18:1978–84. [PubMed: 9528769]

158. Schouten GJ, Vertegaal AC, Whiteside ST, Israel A, Toebes M, Dorsman JC, van der Eb AJ, Zantema A. IkappaB alpha is a target for the mitogen-activated 90 kDa ribosomal S6 kinase. Embo J 1997;16:3133–44. [PubMed: 9214631]

159. Ghoda L, Lin X, Greene WC. The 90-kDa ribosomal S6 kinase (pp90rsk) phosphorylates the N-terminal regulatory domain of IkappaBalpha and stimulates its degradation in vitro. J Biol Chem 1997;272:21281–8. [PubMed: 9261139]

160. Chen RH, Abate C, Blenis J. Phosphorylation of the c-Fos transrepression domain by mitogen-activated protein kinase and 90-kDa ribosomal S6 kinase. Proc Natl Acad Sci USA 1993;90:10952–6. [PubMed: 8248197]

161. Ravi RK, McMahon M, Yangang Z, Williams JR, Dillehay LE, Nelkin BD, Mabry M. Raf-1-induced cell cycle arrest in LNCaP human prostate cancer cells. J Cell Biochem 1999;72:458–69. [PubMed: 10022606]

162. Cucciolla V, Borriello A, Oliva A, Galletti P, Zappia V, Della Ragione F. Resveratrol: from basic science to the clinic. Cell Cycle 2007;6:2495–510. [PubMed: 17726376]

163. Lin HY, Shih A, Davis FB, Tang HY, Martino LJ, Bennett JA, Davis PJ. Resveratrol induced serine phosphorylation of p53 causes apoptosis in a mutant p53 prostate cancer cell line. J Urol 2002;168:748–55. [PubMed: 12131363]

164. Xiao D, Singh SV. Phenethyl isothiocyanate-induced apoptosis in p53-deficient PC-3 human prostate cancer cell line is mediated by extracellular signal-regulated kinases. Cancer Res 2002;62:3615–9. [PubMed: 12097262]

165. Magi-Galluzzi C, Montironi R, Cangi MG, Wishnow K, Loda M. Mitogen-activated protein kinases and apoptosis in PIN. Virchows Arch 1998;432:407–13. [PubMed: 9645439]

166. Wang SI, Parsons R, Ittmann M. Homozygous deletion of the PTEN tumor suppressor gene in a subset of prostate adenocarcinomas. Clin Cancer Res 1998;4:811–5. [PubMed: 9533551]

167. Price DT, Rocca GD, Guo C, Ballo MS, Schwinn DA, Luttrell LM. Activation of extracellular signal-regulated kinase in human prostate cancer. J Urol 1999;162:1537–42. [PubMed: 10492251]

168. Paweletz CP, Charboneau L, Bichsel VE, Simone NL, Chen T, Gillespie JW, Emmert-Buck MR, Roth MJ, Petricoin IE, Liotta LA. Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. Oncogene 2001;20:1981–9. [PubMed: 11360182]

169. Feilotter HE, Nagai MA, Boag AH, Eng C, Mulligan LM. Analysis of PTEN and the 10q23 region in primary prostate carcinomas. Oncogene 1998;16:1743–8. [PubMed: 9582022]
170. Lu X, Nguyen TA, Donehower LA. The Wip1 phosphatase and Md2: cracking the “Wip” on p53 stability. Cell Cycle 2008;7:164–8. [PubMed: 18333294]
171. Dohmesen C, Koeppel M, Dobbelstein M. Specific inhibition of Mdm-2-mediated neddylation by Tip60. Cell Cycle 2008;7:222–31. [PubMed: 18264029]
172. Das S, Boswell SA, Aaronson SA, Lee SW. p53 promoter selction: choosing between life and death. Cell Cycle 2008;7:154–7. [PubMed: 18212532]
173. Lee HJ, Srinivasan D, Coomber D, Lane DP, Vermer CS. Modulation of the p53-MDM2 interaction by phosphorylation of Thr18: a computational study. Cell Cycle 2007;6:2604–11. [PubMed: 17957142]
174. Broude EV, Demidenko ZN, Vivo C, Swift ME, Davis BM, Blagosklonny MV, Roninson IB. p21 CDKN1A is a negative regulator of p53 stability. Cell Cycle 2007;6:1468–71. [PubMed: 17585201]
175. Raver-Shapira N, Oren M. Tiny actors, great roles: microRNAs in p53'service. Cell Cycle 2007;6:2656–61. [PubMed: 17957137]
176. Chen L, Malcolm AJ, Wood KM, Cole M, Variend S, Cullinane C, Pearson AD, Lunec J, Tweddle DA. p53 is nuclear and functional in both undifferentiated and differentiated neuroblastoma. Cell Cycle 2007;6:2685–96. [PubMed: 17912039]
177. Giono LE, Manfredi JJ. Mdm2 plays a positive role as an effector of p53-dependent responses. Cell Cycle 2007;6:2143–7. [PubMed: 17786043]
178. Marusyk A, DeGregori J. Replication stress selects for p53 mutation. Cell Cycle 2007;6:2148–51. [PubMed: 17786047]
179. Melllert H, Sykes SM, Murphy ME, McMahon SB. The ARF/oncogene pathway activates p53 acetylation within the DNA binding domain. Cell Cycle 2007;6:1304–6. [PubMed: 17534149]
180. Efeyan A, Serrano M. p53: guardian of the genome and policeman of the oncogenes. Cell Cycle 2007;6:1006–10. [PubMed: 17457049]
181. Lupi M, Matera G, Natoli C, Colombo V, Ubezio P. The contribution of p53 in the dynamics of cell cycle response to DNA damage interpreted by a mathematical model. Cell Cycle 2007;6:943–950. [PubMed: 17387280]
182. Guo Y, Sklar GN, Borkowski A, Kyprianou N. Loss of the cyclin-dependent kinase inhibitor p27Kip1 protein in human prostate cancer correlates with tumor grade. Clin Cancer Res 1997;3:2269–74. [PubMed: 9815624]
183. Navone NM, Troncoso P, Pisters LL, Goodrow TL, Palmer JL, Nichols WW, von Eschenbach AC, Conti CJ. p53 protein accumulation and gene mutation in the progression of human prostate carcinoma. J Natl Cancer Inst 1993;85:1657–69. [PubMed: 7692074]
184. Phillips SM, Barton CM, Lee SJ, Morton DG, Wallace DM, Lemoine NR, Neoptolemos JP. Loss of the retinoblastoma susceptibility gene (RB1) is a frequent and early event in prostatic tumorigenesis. Br J Cancer 1994;70:1252–7. [PubMed: 7526887]
185. Konishi N, Nakamura M, Kishi M, Nishimine M, Ishida E, Shimada K. Heterogeneous methylation and deletion patterns of the INK4a/ARF locus within prostate carcinomas. Am J Pathol 2002;160:1207–14. [PubMed: 11943705]
186. Badal V, Menendez S, Coomber D, Lane DP. Regulation of the p14ARF promoter by DNA methylation. Cell Cycle 2008;7:112–9. [PubMed: 18196972]
187. Bookstein R, MacGrogan D, Hilsenbeck SG, Sharkey F, Allred DC. p53 is mutated in a subset of advanced-stage prostate cancers. Cancer Res 1993;53:3369–73. [PubMed: 8324747]
188. Voeller HJ, Sugars LY, Pretlow T, Gelmann EP. p53 oncogene mutations in human prostate cancer specimens. J Urol 1994;151:492–5. [PubMed: 7904314]
189. Subler MA, Martin DW, Deb S. Overlapping domains on the p53 protein regulate its transcriptional activation and repression functions. Oncogene 1994;9:1351–9. [PubMed: 8152795]
190. Mallette FA, Ferbeyre G. The DNA damage signaling pathway connects oncogenic stress to cellular senescence. Cell Cycle 2007;6:1831–6. [PubMed: 17671427]

191. Garbe JC, Holst CR, Bassett E, Tlsty T, Stampfer MR. Inactivation of p53 function in cultured human mammary epithelial cells turns the telomere-length dependent senescence barrier from agonescence into crisis. Cell Cycle 2007;6:1927–36. [PubMed: 17671422]
192. Zhang R, Adams PD. Heterochromatin and its relationship to cell senescence and cancer therapy. Cell Cycle 2007;6:784–9. [PubMed: 17377503]
193. Flores ER. The roles of p63 in cancer. Cell Cycle 2007;6:300–4. [PubMed: 17264676]
194. Donner AJ, Hoover JM, Szostek SA, Espinosa JM. Stimulus-specific transcriptional regulation within the p53 network. Cell Cycle 2007;6:2594–8. [PubMed: 17957141]
195. Carroll D, Brugge J, Attardi LD. p63, cell adhesion and survival. Cell Cycle 2007;6:255–61. [PubMed: 17297292]
196. Delacote F, Lopez BS. Importance of the cell cycle phase for the choice of the appropriate DSB repair pathway, for geneome stability maintenance: the trans-S double-strand break repair model. Cell Cycle 2008;7:33–8. [PubMed: 18196958]
197. Kaufmann WK. Initiating the uninitiated: replication of damaged DNA and carcinogenesis. Cell Cycle 2007;6:1460–7. [PubMed: 17582221]
198. Hernandez-Vargas H, von Kobbe C, Sanchez-Estevez C, Julian-Tendero M, Palacios J, Moreno-Bueno G. Inhibition of paclitaxel-induced proteasome activation influences paclitaxel cytotoxicity in breast cancer cells in a sequence-dependent manner. Cell Cycle 2007;6:2662–8. [PubMed: 17912036]
199. Lindsay CR, Scholz A, Morozov VM, Ishov A. Daax shortens mitotic arrest caused by paclitaxel. Cell Cycle 2007;6:1200–4. [PubMed: 17471023]
200. Lafarga V, Cuadrado A, Nebreda AR. P18(Hamlet) mediates different p53-dependent responses to DNA-damaging inducing agents. Cell Cycle 2007;6:2319–22. [PubMed: 17700068]
201. Bartek J, Lukas J, Bartkova J. DNA damage response as an anti-cancer barrier: damage threshold and the concept of ‘conditional haploinsufficiency’. Cell Cycle 2007;6:2344–7. [PubMed: 17700066]
202. Matthew EM, Yen TJ, Dicker DT, Dorsey JF, Yang W, Navaraj A, El-Deiry WS. Replication stress, defective S-phase checkpoint and increased death in Plk2-deficient human cancer cells. Cell Cycle 2007;6:2518–71.
203. Tapia MA, Gonzalez-Navarrete I, Dalmases A, Bosch M, Rodriguez-Fanjul V, Rolfe M, Ross JS, Mezquita J, Mezquita C, Bachs O, Gascon P, Rojo F, Perona R, Rovira A, Albanell J. Inhibition of the canonical IKK/NFkappaB pathway sensitizes human cancer cells to doxorubicin. Cell Cycle 2007;6:2284–92. [PubMed: 17890907]
204. McKeon F, Melino G. Fog of war: the emerging p53 family. Cell Cycle 2007;6:229–32. [PubMed: 17297295]
205. Mazumder S, Plesca D, Almasan A. A Jekyll and hyde role of cyclin E in the genotoxic stress response: switching from cell cycle control to apoptosis regulation. Cell Cycle 2007;6:1437–42. [PubMed: 17581275]
206. Loffler H, Bochtler T, Fritz B, Tews B, Ho AD, Lukas J, Bartek J, Kramer A. DNA damage-induced accumulation of centrosomal Chk1 contributes to its checkpoint function. Cell Cycle 2007;6:2541–8. [PubMed: 17726372]
207. Song H, Xu Y. Gain of function of p53 cancer mutatnts in disrupting critical DNA damage response pathways. Cell Cycle 2007;6:1570–3. [PubMed: 17598983]
208. Lavin MF, Kozlov S. ATM activation and DNA damage response. Cell Cycle 2007;6:931–42. [PubMed: 17457059]
209. Callegari AJ, Kelly TJ. Shedding light on the DNA damage checkpoint. Cell Cycle 2007;6:660–6. [PubMed: 17387276]
210. Blank M, Shiloh Y. Programs for cell death: apoptosis is only one way to go. Cell Cycle 2007;6:686–95. [PubMed: 17361099]
211. Zhang T, Brazhnik P, Tyson JJ. Exploring mechanisms of the DNA-damage response: p53 pulses and their possible relevance to apoptosis. Cell Cycle 2007;6:85–94. [PubMed: 17245126]
212. Kern SE, Pietenpol JA, Thiagalingam S, Seymour A, Kinzler KW, Vogelstein B. Oncogenic forms of p53 inhibit p53-regulated gene expression. Science 1992;256:827–30. [PubMed: 1589764]

213. Scott SL, Earle JD, Gumerlock PH. Functional p53 increases prostate cancer cell survival after exposure to fractionated doses of ionizing radiation. Cancer Res 2003;63:7190–6. [PubMed: 14612513]

214. Chan WM, Siu WY, Lau A, Poon RY. How many mutant p53 molecules are needed to inactivate a tetramer? Mol Cell Biol 2004;24:3536–51. [PubMed: 15060172]

215. Kohn KW, Pommier Y. Molecular interaction map of the p53 and Mdm2 logic elements, which control the Off-On switch of p53 in response to DNA damage. Biochem Biophys Res Commun 2005;331:816–27. [PubMed: 15865937]

216. Coutts AS, La Thangue NB. Mdm2 widens its repertoire. Cell Cycle 2007;6:827–9. [PubMed: 17377491]

217. Lindstrom MS, Deisenroth C, Zhang Y. Putting a finger on growth surveillance: insight into MDM2 zinc finger-ribosomal protein interactions. Cell Cycle 2007;6:434–7. [PubMed: 17329973]

218. Gilkes DM, Chen J. Distinct roles of MDMX in the regulation of p53 response to ribosomal stress. Cell Cycle 2007;6:151–5. [PubMed: 17327702]

219. Linares LK, Hengstermann A, Ciechanover A, Muller S, Scheffner M. HdmX stimulates Hdm2-mediated ubiquitination and degradation of p53. Proc Natl Acad Sci USA 2003;100:12009–14. [PubMed: 14507994]

220. Fuchs SY, Adler V, Buschmann T, Wu X, Ronai Z. Mdm2 association with p53 targets its ubiquitination. Oncogene 1998;17:2543–7. [PubMed: 9824166]

221. Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell 1992;69:1237–45. [PubMed: 1535557]

222. Gu J, Kawai H, Nie L, Kitao H, Wiederschain D, Jochemsen AG, Parant J, Lozano G, Yuan ZM. Mutual dependence of MDM2 and MDMX in their functional inactivation of p53. J Biol Chem 2002;277:19251–4. [PubMed: 11953423]

223. Chehab NH, Malikzay A, Stavridi ES, Halazonetis TD. Phosphorylation of Ser-20 mediates stabilization of human p53 in response to DNA damage. Proc Natl Acad Sci USA 1999;96:13777–82. [PubMed: 10570149]

224. Tibbetts RS, Brumbaugh KM, Williams JM, Sarkaria JN, Cliby WA, Shieh SY, Taya Y, Prives C, Abraham RT. A role for ATR in the DNA damage-induced phosphorylation of p53. Genes Dev 1999;13:152–7. [PubMed: 9925639]

225. Kuerbitz SJ, Plunkett BS, Walsh WV, Kastan MB. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc Natl Acad Sci USA 1992;89:7491–5. [PubMed: 1323840]

226. Helt CE, Cliby WA, Keng PC, Bambara RA, O'Reilly MA. Ataxia telangiectasia mutated (ATM) and ATM and Rad3-related protein exhibit selective target specificities in response to different forms of DNA damage. J Biol Chem 2005;280:1186–92. [PubMed: 15533933]

227. Hurley PJ, Bunz F. ATM and ATR: components of an integrated circuit. Cell Cycle 2007;6:414–7. [PubMed: 17312392]

228. Tanaka T, Halicka HD, Traganos F, Seiter K, Darzynkiewicz Z. Induction of ATM activation, histone H2AX phosphorylation and apoptosis by etoposide: relation to cell cycle phase. Cell Cycle 2007;6:371–6. [PubMed: 17297310]

229. Milner J, Medcalf EA, Cook AC. Tumor suppressor p53: analysis of wild-type and mutant p53 complexes. Mol Cell Biol 1991;11:12–9. [PubMed: 1986215]

230. de Vries A, Flores ER, Miranda B, Hsieh HM, van Oostrom CT, Sage J, Jacks T. Targeted point mutations of p53 lead to dominant-negative inhibition of wild-type p53 function. Proc Natl Acad Sci USA 2002;99:2948–53. [PubMed: 11867759]

231. Petitjean A, Achatz MI, Borresen-Dale AL, Hainaut P, Olivier M. TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. Oncogene 2007;26:2157–65. [PubMed: 17401424]

232. Barak Y, Juven T, Haffner R, Oren M. mdm2 expression is induced by wild type p53 activity. EMBO J 1993;12:461–8. [PubMed: 8440237]

233. Song H, Hollstein M, Xu Y. p53 gain-of-function cancer mutants induce genetic instability by inactivating ATM. Nat Cell Biol 2007;9:573–80. [PubMed: 17417627]

234. Ongusaha PP, Kim JI, Fang L, Wong TW, Yancopoulos GD, Aaronson SA, Lee SW. p53 induction and activation of DDR1 kinas counteract p53-mediated apoptosis and influence p53 regulation through a positive feedback loop. EMBO J 2003;22:1289–301. [PubMed: 12628922]

235. Das S, Ongusaha PP, Yang YS, Park JM, Aaronson SA, Lee SW. Discoidin domain receptor receptor 1 receptor tyrosine kinase induces cyclooxygenase-2 and promotes chemoresistance through nuclear factor-kappa B pathway activation. Cancer Res 2006;66:8123–30. [PubMed: 16912190]

236. Shimada K, Nakamura M, Ishida E, Higuchi T, Yamamoto H, Tsujikawa K, Konishi N. Prostate cancer antigen-1 contributes to cell survival and invasion through discoidin receptor 1 in human prostate cancer. Cancer Sci 2008;99:39–45. [PubMed: 17970783]

237. Singh S, Upadhyay AK, Ajay AK, Bhat MK. p53 regulates ERK activation in carboplatin induced apoptosis in cervical carcinoma: A novel target of p53 in apoptosis. FEBS Lett 2007;581:289–95. [PubMed: 17208232]

238. Ogawara Y, Kishishita S, Obata T, Isazawa Y, Suzuki T, Tanaka K, Masuyama Y, Gotoh Y. Akt enhances Mdm2-mediated ubiquitination and degradation of p53. J Biol Chem 2002;277:21843–50. [PubMed: 11923280]

239. Freeman DJ, Li A, Wei G, Li HH, Kertesz N, Lesche R, Whale AD, Martinez-Diaz H, Rozengurt N, Cardiff RD, Liu X, Wu H. PTEN tumor suppressor regulates p53 protein levels and activity through phosphatase-dependent and -independent mechanisms. Cancer Cell 2003;3:117–30. [PubMed: 12620407]

240. Chang CJ, Mulholland DJ, Valamehr B, Mosessian S, Sellers WR, Wu H. PTEN nuclear localization is regulated by oxidative stress and mediates p53-dependent tumor suppression. Mol Cell Biol. 2008In Press

241. Whelan JT, Forbes SL, Bertrand FE. CBF-1 (RBP-J kappa) binds to the PTEN promoter and regulates PTEN gene expression. Cell Cycle 2007;6:80–4. [PubMed: 17245125]

242. Li Y, Guessous F, Kwon S, Kumar M, Ibidapo O, Fuller L, Johnson E, Lai B, Hussaini I, Bao Y, Laterra J, Schiff D, Abounader R. PTEN has tumor-promoting properties in the setting of gain-of-function p53 mutations. Cancer Res 2008;68:1723–31. [PubMed: 18339852]

243. Shi XB, Nesslinger NJ, Deitch AD, Gumerlock PH, deVere White RW. Complex functions of mutant p53 alleles from human prostate cancer. Prostate 2002;51:59–72. [PubMed: 11920959]

244. Algan O, Stobbe CC, Helt AM, Hanks GE, Chapman JD. Radiation inactivation of human prostate cancer cells: the role of apoptosis. Radiat Res 1996;146:267–75. [PubMed: 8752304]

245. Cao C, Shinohara ET, Subhawong TK, Geng L, Woon Kim K, Albert JM, Hallahan DE, Lu B. Radiosensitization of lung cancer by nutlin, an inhibitor of murine double minute 2. Mol Cancer Ther 2006;5:411–7. [PubMed: 16505116]

246. Raffoul JJ, Wang Y, Kucuk O, Forman JD, Sarkar FH, Hillman GG. Genistein inhibits radiation-induced activation of NFkappaB in prostate cancer cells promoting apoptosis and G_2/M cell cycle arrest. BMC Cancer 2006;6:107. [PubMed: 16640785]

247. Rosen EM, Fan S, Rockwell S, Goldberg ID. The molecular and cellular basis of radiosensitivity: implications for understanding how normal tissues and tumors respond to therapeutic radiation. Cancer Invest 1999;17:56–72. [PubMed: 10999050]

248. Sturgeon CM, Roberge M. G_2 checkpoint kinase inhibitors exert their radiosensitizing effects prior to the G_2/M transition. Cell Cycle 2007;6:572–5. [PubMed: 17351346]

249. Cao C, Subhawong T, Albert JM, Subhawong TK, Shinohara ET, Albert JM, Ling G, Cao C, Gi YJ, Lu B. Inhibition of mammalian target of rapamycin or apoptotic pathway induces autophagy and radiosensitizes PTEN null prostate cancer cells. Cancer Res 2006;66:10040–7. [PubMed: 17047067]

250. Lehmann BD, McCubrey JA, Jefferson HS, Paine MS, Chappell WH, Terrian DM. A dominant role for p53-dependent cellular senescence in radiosensitization of human prostate cancer cells. Cell Cycle 2007;6:595–605. [PubMed: 17351335]

251. Crighton D, Wilkinson S, O'Prey J, Syed N, Smith P, Harrison PR, Gasco M, Garrone O, Crook T, Ryan KM. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. Cell 2006;126:121–34. [PubMed: 16839881]

252. Ventura A, Kirsch DG, McLaughlin ME, Tuveson DA, Grimm J, Lintault L, Newman J, Reczek EE, Weissleder R, Jacks T. Restoration of p53 function leads to tumour regression in vivo. Nature 2007;445:661–5. [PubMed: 17251932]
253. Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V, Cordon-Cardo C, Lowe SW. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature 2007;445:656–60. [PubMed: 17251933]
254. Janicke RU, Sohn D, Essmann F, Schulze-Osthoff K. The multiple battles fought by anti-apoptotic p21. Cell Cycle 2007;6:407–13. [PubMed: 17312393]
255. Dotto GP. p21(WAF1/Cip1): more than a break to the cell cycle? Biochim Biophys Acta 2000;1471:43–56.
256. Narita M, Nunez S, Heard E, Narita M, Lin AW, Hearn SA, Spector DL, Hannon GJ, Lowe SW. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 2003;113:703–16. [PubMed: 12809602]
257. Dai CY, Enders GH. p16 INK4a can initiate an autonomous senescence program. Oncogene 2000;19:1613–22. [PubMed: 10763818]
258. Sugrue MM, Shin DY, Lee SW, Aaronson SA. Wild-type p53 triggers a rapid senescence program in human tumor cells lacking functional p53. Proc Natl Acad Sci USA 1997;94:9648–53. [PubMed: 9275177]
259. Fang L, Igarashi M, Leung J, Sugrue MM, Lee SW, Aaronson SA. $p21^{Waf1/Cip1/Sdi1}$ induces permanent growth arrest with markers of replicative senescence in human tumor cells lacking functional p53. Oncogene 1999;18:2789–97. [PubMed: 10362249]
260. Beausejour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, Campisi J. Reversal of human cellular senescence: roles of the p53 and p16 pathways. Embo J 2003;22:4212–22. [PubMed: 12912919]
261. Nielsen SJ, Schneider R, Bauer UM, Bannister AJ, Morrison A, O'Carroll D, Firestein R, Cleary M, Jenuwein T, Herrera RE, Kouzarides T. Rb targets histone H3 methylation and HP1 to promoters. Nature 2001;412:561–5. [PubMed: 11484059]
262. Vandel L, Nicolas E, Vaute O, Ferreira R, Ait-Si-Ali S, Trouche D. Transcriptional repression by the retinoblastoma protein through the recruitment of a histone methyltransferase. Mol Cell Biol 2001;21:6484–94. [PubMed: 11533237]
263. Hu B, Gilkes DM, Farooqi B, Sebti SM, Chen J. MDMX overexpression prevents p53 activation by the MDM2 inhibitor Nutlin. J Biol Chem 2006;281:33030–5. [PubMed: 16905541]
264. Saric T, Brkanac Z, Troyer DA, Padalecki SS, Sarosdy M, Williams K, Abadesco L, Leach RJ, O'Connell P. Genetic pattern of prostate cancer progression. Int J Cancer 1999;81:219–24. [PubMed: 10188722]
265. Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, Koutcher JA, Scher HI, Ludwig T, Gerald W, Cordon-Cardo C, Pandolfi PP. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature 2005;436:725–30. [PubMed: 16079851]
266. Hill R, Song Y, Cardiff RD, Van Dyke T. Heterogeneous tumor evolution initiated by loss of pRb function in a preclinical prostate cancer model. Cancer Res 2005;65:10243–54. [PubMed: 16288012]
267. Harris KA, Reese DM. Treatment options in hormone-refractory prostate cancer: current and future approaches. Drugs 2001;61:2177–92. [PubMed: 11772129]
268. Waselenko JK, Dawson NA. Management of progressive metastatic prostate cancer. Oncology (Huntingt) 1997;11:1551–60. [PubMed: 9348559]
269. Hussain A, Dawson N. Management of advanced/metastatic prostate cancer. Oncology (Huntingt) 2000;14:1677–88. [PubMed: 11204373]
270. Gulley J, Dahut WL. Chemotherapy for prostate cancer: finally an advance! Am J Ther 2004;11:288–94. [PubMed: 15266221]
271. Gulley J, Dahut W. Novel clinical trials in androgen-independent prostate cancer. Clin Prostate Cancer 2002;1:51–7. [PubMed: 15046713]
272. Retter AS, Gulley JL, Dahut WL. Novel therapeutic strategies in prostate cancer. Cancer Biol Ther 2004;3:371–6. [PubMed: 14726702]

273. Comstock CES, Knudsen KE. The complex role of AR Signaling after cytotoxic insult: implications for cell cycle-based chemotherapeutics. Cell Cycle 2007;6:1307–13. [PubMed: 17568191]

274. Vander Griend DJ, Litvinov IV, Isaacs JT. Stablizing androgen receptor in mitosis inhibits prostate cancer proliferation. Cell Cycle 2007;6:647–51. [PubMed: 17387277]

275. Harris KA, Reese DM. Treatment options in hormone-refractory prostate cancer: current and future approaches. Drugs 2001;61:2177–92. [PubMed: 11772129]

276. Kruh GD. Introduction to resistance to anticancer agents. Oncogene 2003;22:7262–4. [PubMed: 14576836]

277. Garcia R, Franklin RA, McCubrey JA. EGF induces cell motility and multi-drug resistance gene expression in breast cancer cells. Cell Cycle 2006;5:2820–6. [PubMed: 17172846]

278. Garcia R, Franklin RA, McCubrey JA. Cell death of MCF-7 human breast cancer cells induced by EGFR activation in the absence of other growth factors. Cell Cycle 2006;5:1840–6. [PubMed: 16861904]

279. Grunwald V, DeGraffenried L, Russel D, Friedrichs WE, Ray RB, Hidalgo M. Inhibitors of mTOR reverse doxorubicin resistance conferred by PTEN status in prostate cancer cells. Cancer Res 2002;62:6141–5. [PubMed: 12414639]

280. Pfeil K, Eder IE, Putz T, Ramoner R, Culig Z, Ueberall F, Bartsch G, Klocker H. Long-term androgen-ablation causes increased resistance to PI3K/Akt pathway inhibition in prostate cancer cells. Prostate 2004;58:259–68. [PubMed: 14743465]

281. Lee JT Jr, Steelman LS, McCubrey JA. Phosphatidylinositol 3'-kinase activation leads to multidrug resistance protein-1 expression and subsequent chemoresistance in advanced prostate cancer cells. Cancer Res 2004;64:8397–404. [PubMed: 15548710]

282. Lee C, Kim JS, Waldman T. Activated PI3K signalling as an endogenous inducer of p53 in human cancer. Cell Cycle 2007;6:394–6. [PubMed: 17329971]

283. Skladanowski A, Bozko P, Sabisz M, Larsen AK. Dual inhibition of PI3K/Akt signaling and the DNA damage checkpoint in p53-deficient cells with strong survival signaling: implications for cancer therapy. Cell Cycle 2007;6:2268–75. [PubMed: 17890906]

284. Shukla S, Gupta S. Apigenin-induced cell cycle arrest is mediated by modulation of MAPK, PI3K-Akt, and loss of cyclin D1 associated retinoblastoma dephosphorylation in human prostate cancer cells. Cell Cycle 2007;6:1102–14. [PubMed: 17457054]

285. Liu Z, Roberts TM. Human tumor mutants in the p110alpha subunit of PI3K. Cell Cycle 2006;5:675–7. [PubMed: 16627990]

286. Nielsen D, Maare C, Skovsgaard T. Cellular resistance to anthracyclines. Gen Pharmacol 1996;27:251–5. [PubMed: 8919638]

287. Kantoff PW, Halabi S, Conaway M, Picus J, Kirshner J, Hars V, Trump D, Winer EP, Vogelzang NJ. Hydrocortisone with or without mitoxantrone in men with hormone-refractory prostate cancer: results of the cancer and leukemia group B 9182 study. J Clin Oncol 1999;17:2506–13. [PubMed: 10561316]

288. Tannock IF, Osoba D, Stockler MR, Ernst DS, Neville AJ, Moore MJ, Armitage GR, Wilson JJ, Venner PM, Coppin CM, Murphy KC. Chemotherapy with mitoxantrone plus prednisone or prednisone alone for symptomatic hormone-resistant prostate cancer: a Canadian randomized trial with palliative end points. J Clin Oncol 1996;14:1756–64. [PubMed: 8656243]

289. Orr GA, Verdier-Pinard P, McDaid H, Horwitz SB. Mechanisms of Taxol resistance related to microtubules. Oncogene 2003;22:7280–95. [PubMed: 14576838]

290. Horwitz SB. Taxol (paclitaxel): mechanisms of action. Ann Oncol 1994;6:3–6.

291. Obasaju C, Hudes GR. Paclitaxel and docetaxel in prostate cancer. Hematol Oncol Clin North Am 2001;15:525–45. [PubMed: 11525295]

292. Hudes GR, Obasaju C, Chapman A, Gallo J, McAleer C, Greenberg R. Phase I study of paclitaxel and estramustine: preliminary activity in hormone-refractory prostate cancer. Semin Oncol 1995;22:6–11. [PubMed: 7597435]

293. Gilligan T, Kantoff PW. Chemotherapy for prostate cancer. Urology 2002;60:94–100. [PubMed: 12231060]

294. Pathan N, Aime-Sempe C, Kitada S, Haldar S, Reed JC. Microtubule-targeting drugs induce Bcl-2 phosphorylation and association with Pin1. Neoplasia 2001;3:70–9. [PubMed: 11326318]

295. Haldar S, Chintapalli J, Croce CM. Taxol induces bcl-2 phosphorylation and death of prostate cancer cells. Cancer Res 1996;56:1253–5. [PubMed: 8640809]

296. Tew KD, Stearns ME. Estramustine—a nitrogen mustard/steroid with antimicrotubule activity. Pharmacol Ther 1989;43:299–319. [PubMed: 2682681]

297. Panda D, Miller HP, Islam K, Wilson L. Stabilization of microtubule dynamics by estramustine by binding to a novel site in tubulin: a possible mechanistic basis for its antitumor action. Proc Natl Acad Sci USA 1997;94:10560–4. [PubMed: 9380674]

298. Perry CM, McTavish D. Estramustine phosphate sodium. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in prostate cancer. Drugs Aging 1995;7:49–74. [PubMed: 7579781]

299. Canil CM, Tannock IF. Is there a role for chemotherapy in prostate cancer? Br J Cancer 2004;91:1005–11. [PubMed: 15150548]

300. Lee C, Kim JS, Waldman T. Activated PI3K signaling as an endogenous inducer of p53 in human cancer. Cell Cycle 2007;6:394–6. [PubMed: 17329971]

301. Gagnon V, Mathieu I, Sexton E, Leblanc K, Asselin E. AKT involvement in cisplatin chemoresistance of human uterine cancer cells. Gynecol Oncol 2004;94:785–95. [PubMed: 15350374]

302. Foster DA. Targeting mTOR-mediated survival signals in anticancer therapeutic strategies. Expert Rev Anticancer Ther 2004;4:691–701. [PubMed: 15270672]

303. Zhao Y, You H, Yang Y, Wei L, Zhang X, Yao L, Fan D, Yu Q. Distinctive regulation and function of PI 3K/Akt and MAPKs in doxorubicin-induced apoptosis of human lung adenocarcinoma cells. J Cell Biochem 2004;91:621–32. [PubMed: 14755690]

304. She QB, Solit D, Basso A, Moasser MM. Resistance to gefitinib in PTEN-null HER-over-expressing tumor cells can be overcome through restoration of PTEN function or pharmacologic modulation of constitutive phosphatidylinositol 3'-kinase/Akt pathway signaling. Clin Cancer Res 2003;9:4340–6. [PubMed: 14555504]

305. Knuefermann C, Lu Y, Liu B, Jin W, Liang K, Wu L, Schmidt M, Mills GB, Mendelsohn J, Fan Z. HER2/PI-3K/Akt activation leads to a multidrug resistance in human breast adeno-carcinoma cells. Oncogene 2003;22:3205–12. [PubMed: 12761490]

306. Jin W, Wu L, Liang K, Liu B, Lu Y, Fan Z. Roles of the PI-3K and MEK pathways in Ras-mediated chemoresistance in breast cancer cells. Br J Cancer 2003;89:185–91. [PubMed: 12838322]

307. Pfeil K, Eder IE, Putz T, Ramoner R, Culig Z, Ueberall F, Bartsch G, Klocker H. Long-term androgen-ablation causes increased resistance to PI3K/Akt pathway inhibition in prostate cancer cells. Prostate 2004;58:259–68. [PubMed: 14743465]

308. Zalcberg J, Hu XF, Slater A, Parisot J, El-Osta S, Kantharidis P, Chou ST, Parkin JD. MRP1 not MDR1 gene expression is the predominant mechanism of acquired multidrug resistance in two prostate carcinoma cell lines. Prostate Cancer Prostatic Dis 2000;3:66–75. [PubMed: 12497102]

309. Wang Q, Beck WT. Transcriptional suppression of multidrug resistance-associated protein (MRP) gene expression by wild-type p53. Cancer Res 1998;58:5762–9. [PubMed: 9865734]

310. Sullivan GF, Yang JM, Vassil A, Yang J, Bash-Babula J, Hait WN. Regulation of expression of the multidrug resistance protein MRP1 by p53 in human prostate cancer cells. J Clin Invest 2000;105:1261–7. [PubMed: 10792001]

311. Tiwari G, Sakaue H, Pollack JR, Roth RA. Gene expression profiling in prostate cancer cells with Akt activation reveals Fra-1 as an Akt-inducible gene. Mol Cancer Res 2003;1:475–84. [PubMed: 12692267]

312. Berges RR, Vukanovic J, Epstein JI, CarMichel M, Cisek L, Johnson DE, Veltri RW, Walsh PC, Isaacs JT. Implication of cell kinetic changes during the progression of human prostatic cancer. Clin Cancer Res 1995;1:473–80. [PubMed: 9816006]

313. Horiatis D, Wang Q, Pinski J. A new screening system for proliferation-independent anti-cancer agents. Cancer Lett 2004;210:119–24. [PubMed: 15172129]

314. Mandic A, Viktorsson K, Heiden T, Hansson J, Shoshan MC. The MEK1 inhibitor PD98059 sensitizes C8161 melanoma cells to cisplatin-induced apoptosis. Melanoma Res 2001;11:11–9. [PubMed: 11254111]

315. Hayakawa J, Ohmichi M, Kurachi H, Ikegami H, Kimura A, Matsuoka T, Jikihara H, Mercola D, Murata Y. Inhibition of extracellular signal-regulated protein kinase or c-Jun N-terminal protein kinase cascade, differentially activated by cisplatin, sensitizes human ovarian cancer cell line. J Biol Chem 1999;274:31648–54. [PubMed: 10531373]

316. Lee M, Koh WS, Han SS. Downregulation of Raf-1 kinase is associated with paclitaxel resistance in human breast cancer MCF-7/Adr cells. Cancer Lett 2003;193:57–64. [PubMed: 12691824]

317. Yeh PY, Chuang SE, Yeh KH, Song YC, Chang LL, Cheng AL. Phosphorylation of p53 on Thr55 by ERK2 is necessary for doxorubicin-induced p53 activation and cell death. Oncogene 2004;23:3580–8. [PubMed: 15116093]

318. Yeh PY, Chuang SE, Yeh KH, Song YC, Ea CK, Cheng AL. Increase of the resistance of human cervical carcinoma cells to cisplatin by inhibition of the MEK to ERK signaling pathway partly via enhancement of anticancer drug-induced NFkappaB activation. Biochem Pharmacol 2002;63:1423–30. [PubMed: 11996883]

319. Wang X, Martindale JL, Holbrook NJ. Requirement for ERK activation in cisplatin-induced apoptosis. J Biol Chem 2000;275:39435–43. [PubMed: 10993883]

320. Lee JT Jr, Steelman LS, McCubrey JA. Modulation of Raf/MEK/ERK kinase activity does not affect the chemoresistance profile of advanced prostate cancer cells. Int J Oncol 2005;26:1637–44. [PubMed: 15870880]

321. Choi BK, Choi CH, Oh HL, Kim YK. Role of ERK Activation in Cisplatin-Induced Apoptosis in A172 Human Glioma Cells. Neurotoxicology 2004;25:915–24. [PubMed: 15474610]

322. Davis JM, Navolanic PM, Weinstein-Oppenheimer CR, Steelman LS, Hu W, Konopleva M, Blagosklonny MV, McCubrey JA. Raf-1 and Bcl-2 induce distinct and common pathways that contribute to breast cancer drug resistance. Clin Cancer Res 2003;9:1161–70. [PubMed: 12631622]

323. Weinstein-Oppenheimer CR, Henriquez-Roldan CF, Davis JM, Navolanic PM, Saleh OA, Steelman LS, Franklin RA, Robinson PJ, McMahon M, McCubrey JA. Role of the Raf signal transduction cascade in the in vitro resistance to the anticancer drug doxorubicin. Clin Cancer Res 2001;7:2898–907. [PubMed: 11555608]

324. Yeh PY, Chuang SE, Yeh KH, Song YC, Cheng AL. Nuclear extracellular signal-regulated kinase 2 phosphorylates p53 at Thr55 in response to doxorubicin. Biochem Biophys Res Commun 2001;284:880–6. [PubMed: 11409876]

325. Bacus SS, Gudkov AV, Lowe M, Lyass L, Yung Y, Komarov AP, Keyomarsi K, Yarden Y, Seger R. Taxol-induced apoptosis depends on MAP kinase pathways (ERK and p38) and is independent of p53. Oncogene 2001;20:147–55. [PubMed: 11313944]

326. Bohnke A, Westphal F, Schmidt A, El-Awady RA, Dahm-Daphi J. Role of p53 mutations, protein function and DNA damage for the radiosensitivity of human tumour cells. Int J Radiat Biol 2004;80:53–63. [PubMed: 14761850]

327. Kreisberg JI, Malik SN, Prihoda TJ, Bedolla RG, Troyer DA, Kreisberg S, Ghosh PM. Phosphorylation of Akt (Ser473) is an excellent predictor of poor clinical outcome in prostate cancer. Cancer Res 2004;64:5232–6. [PubMed: 15289328]

328. Ghosh PM, Malik S, Bedolla R, Kreisberg JI. Akt in prostate cancer: possible role in androgen-independence. Curr Drug Metab 2003;4:487–96. [PubMed: 14683476]

329. Lee JT, Steelman LS, Chappell W, McCubrey JA. Akt inactivates ERK causing decreased response to chemotherapeutic drugs in advanced CaP cells. Cell Cycle 2008;7In Press

330. Zimmermann S, Moelling K. Phosphorylation and regulation of Raf by Akt (protein kinase B). Science 1999;286:1741–4. [PubMed: 10576742]

331. Mandic A, Viktorsson K, Heiden T, Hansson J, Shoshan MC. The MEK1 inhibitor PD98059 sensitizes C8161 melanoma cells to cisplatin-induced apoptosis. Melanoma Res 2001;11:11–9. [PubMed: 11254111]

332. Schweyer S, Soruri A, Meschter O, Heintze A, Zschunke F, Miosge N, Thelen P, Schlott T, Radzun HJ, Fayyazi A. Cisplatin-induced apoptosis in human malignant testicular germ cell lines depends on MEK/ERK activation. Br J Cancer 2004;91:589–98. [PubMed: 15266324]

333. Wei SQ, Sui LH, Zheng JH, Zhang GM, Kao YL. Role of ERK1/2 kinase in cisplatin-induced apoptosis in human ovarian carcinoma cells. Chin Med Sci J 2004;19:125–9. [PubMed: 15250250]

334. Cooperberg MR, Broering JM, Litwin MS, Lubeck DP, Mehta SS, Henning JM, Carroll PR. CaPSURE Investigators. The contemporary management of prostate cancer in the United States: lessons from the cancer of the prostate strategic urologic research endeavor (CapSURE), a national disease registry. J Urol 2004;171:1393–401. [PubMed: 15017184]

335. Nichol AM, Warde P, Bristow RG. Optimal treatment of intermediate-risk prostate carcinoma with radiotherapy: clinical and translational issues. Cancer 2005;104:891–905. [PubMed: 16007687]

336. Ward JF, Blute ML. Use and timing of radiotherapy in high-risk prostate cancer. Jama 2004;291:2817–8. [PubMed: 15199027]

337. Kupelian PA, Potters L, Khuntia D, Ciezki JP, Reddy CA, Reuther AM, Carlson TP, Klein EA. Radical prostatectomy, external beam radiotherapy <72 Gy, external beam radiotherapy ≥72 Gy, permanent seed implantation, or combined seeds/external beam radiotherapy for stage T1-T2 prostate cancer. Int J Radiat Oncol Biol Phys 2004;58:25–33. [PubMed: 14697417]

338. Zelefsky MJ, Ben-Porat L, Chan HM, Fearn PA, Venkatraman ES. Evaluation of postradiotherapy PSA patterns and correlation with 10-year disease free survival outcomes for prostate cancer. Int J Radiat Oncol Biol Phys 2006;66:382–8. [PubMed: 16965990]

339. Pollack A, Zagars GK, Starkschall G, Antolak JA, Lee JJ, Huang E, von Eschenbach AC, Kuban DA, Rosen I. Prostate cancer radiation dose response: results of the M. D. Anderson phase III randomized trial. Int J Radiat Oncol Biol Phys 2002;53:1097–105. [PubMed: 12128107]

340. Hermann PC, Huber SL, Heeschen C. Metastatic cancer stem cells: a new target for anti-cancer therapy? Cell Cycle 2008;7:188–93. [PubMed: 18256530]

341. Borst P, Jonkers J, Rottenberg S. What makes tumors multidrug resistant? Cell Cycle 2008;7:2782–7.

342. Miller JP, Yeh N, Vidal A, Koff A. Interweaving the cell cycle machinery with cell differentiation. Cell Cycle 2007:2932–8. [PubMed: 18000404]

343. Rapp UR, Ceteci F, Schreck R. Oncogene-induced plasticity and cancer stem cells. Cell Cycle 2008;7:45–51. [PubMed: 18196970]

344. Cambell L, Polyak K. Breast tumor heterogeneity: cancer stem cells or clonal evolution? Cell Cycle 2007;6:2332–8. [PubMed: 17786053]

345. Ohm JE, Baylin SB. Stem cell chromatin patterns: an instructive mechanism for DNA hypermethylation? Cell Cycle 2007;6:1040–3. [PubMed: 17457052]

346. Finlan LE, Hupp TR. p63: the phantom of the tumor suppressor. Cell Cycle 2007;6:1062–71. [PubMed: 17426453]

347. Steel GG. The case against apoptosis. Acta Oncol 2001;40:968–75. [PubMed: 11845962]

348. Shay JW, Roninson IB. Hallmarks of senescence in carcinogenesis and cancer therapy. Oncogene 2004;23:2919–33. [PubMed: 15077154]

349. Honda R, Tanaka H, Yasuda H. Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. FEBS Lett 1997;420:25–7. [PubMed: 9450543]

350. Khor LY, Desilvio M, Al-Saleem T, Hammond ME, Grignon DJ, Sause W, Pilepich M, Okunieff P, Sandler H, Pollack A. Radiation Therapy Oncology Group. MDM2 as a predictor of prostate carcinoma outcome: an analysis of Radiation Therapy Oncology Group Protocol 8610. Cancer 2005;104:962–7. [PubMed: 16007688]

351. Mu Z, Hachem P, Agrawal S, Pollack A. Antisense MDM2 sensitizes prostate cancer cells to androgen deprivation, radiation and the combination. Int J Radiat Oncol Biol Phys 2004;58:336–43. [PubMed: 14751501]

352. Zhang R, Wang H, Agrawal S. Novel antisense anti-MDM2 mixed-backbone oligonucleotides: proof of principle, in vitro and in vivo activities, and mechanisms. Curr Cancer Drug Targets 2005;5:43–9. [PubMed: 15720188]

353. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, Fotouhi N, Liu EA. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science 2004;303:844–8. [PubMed: 14704432]

354. Tovar C, Rosinski J, Filipovic Z, Higgins B, Kolinsky K, Hilton H, Zhao X, Vu BT, Qing W, Packman K, Myklebost O, Heimbrook DC, Vassilev LT. Small-molecule MDM2 antagonists reveal aberrant p53 signaling in cancer: implications for therapy. Proc Natl Acad Sci USA 2006;103:1888–93. [PubMed: 16443686]

355. Maddison LA, Sutherland BW, Barrios RJ, Greenberg NM. Conditional deletion of Rb causes early stage prostate cancer. Cancer Res 2004;64:6018–25. [PubMed: 15342382]

356. Mayo LD, Donner DB. A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. Proc Natl Acad Sci USA 2001;98:11598–603. [PubMed: 11504915]

357. Festuccia C, Gravina GL, Muzi P, Millimaggi D, Dolo V, Vicentini C, Bologna M. Akt downmoduclation induces apoptosis of human prostate cancer cells and synergizes with EGFR tyrosine kinase inhibitors. Prostate. 2008In Press

358. Zhou BP, Liao Y, Xia W, Zou Y, Spohn B, Hung MC. HER-2/neu induces p53 ubiquitination via Akt-mediated MDM2 phosphorylation. Nat Cell Biol 2001;3:973–82. [PubMed: 11715018]

359. Kim JS, Lee C, Bonifant CL, Ressom H, Waldman T. Activation of p53-dependent growth suppression in human cells by mutations in PTEN or PIK3CA. Mol Cell Biol 2007;27:662–77. [PubMed: 17060456]

360. Lee S, Lee HS, Baek M, Lee DY, Bang YJ, Cho HN, Lee YS, Ha JH, Kim HY, Jeoung DI. MAPK signaling is involved in camptothecin-induced cell death. Mol Cells 2002;14:348–54. [PubMed: 12521296]

361. Donnelly JG. Pharmacogenetics in cancer chemotherapy: balancing toxicity and response. Ther Drug Monit 2004;26:231–5. [PubMed: 15228171]

362. El Sheikh SS, Domin J, Abel P, Stamp G, Lalani el N. Phosphorylation of both EGFR and ErbB2 is a reliable predictor of prostate cancer cell proliferation in response to EGF. Neoplasia 2004;6:846–53. [PubMed: 15720812]

363. Shelton JG, Blalock WL, White ER, Steelman LS, McCubrey JA. Ability of the activated PI3K/Akt oncoproteins to synergize with MEK1 and induce cell cycle progression and abrogate the cytokine-dependence of hematopoietic cells. Cell Cycle 2004;3:503–12. [PubMed: 15004527]

364. McCubrey JA, Steelman LS, Blalock WL, Lee JT, Moye PW, Chang F, Pearce M, Shelton JG, White MK, Franklin RA, Pohnert SC. Synergistic effects of pi3k/akt on abrogation of cytokine-dependency induced by oncogenic raf. Adv Enzyme Regul 2001;41:289–323. [PubMed: 11384752]

365. McCubrey JA, Lee JT, Steelman LS, Blalock WL, Moye PW, Chang F, Pearce M, Shelton JG, White MK, Franklin RA, Pohnert SC. Interactions between the PI3K and Raf signaling pathways can result in the transformation of hematopoietic cells. Cancer Detect Prev 2001;25:375–93. [PubMed: 11531015]

366. Blalock WL, Navolanic PM, Steelman LS, Shelton JG, Moye PW, Lee JT, Franklin RA, Mirza A, McMahon M, White MK, McCubrey JA. Requirement for the PI3K/Akt pathway in MEK1-mediated growth and prevention of apoptosis: identification of an Achilles heel in leukemia. Leukemia 2003;17:1058–67. [PubMed: 12764369]

367. Shelton JG, Steelman LS, White ER, McCubrey JA. Synergy between PI3K/Akt and Raf/MEK/ERK Pathways in IGF-1R Mediated Cell Cycle Progression and Prevention of Apoptosis in Hematopoietic Cells. Cell Cycle 2004;3:372–9. [PubMed: 14726697]

368. Shelton JG, Steelman LS, Abrams SL, White ER, Akula SM, Franklin RA, Bertrand FE, McMahon M, McCubrey JA. Conditional EGFR promotes cell cycle progression and prevention of apoptosis in the absence of autocrine cytokines. Cell Cycle 2005;4:822–30. [PubMed: 15917660]

369. Shelton JG, Steelman LS, Abrams SL, White ER, Akula SM, Bertrand FE, Franklin RA, McCubrey JA. Effects of endogenous epidermal growth factor receptor signaling on DNA synthesis and ERK activation in a cytokine-dependent hematopoietic cell line. Cell Cycle 2005;4:818–21. [PubMed: 15908804]

370. Shimizu Y, Segawa T, Inoue T, Shiraishi T, Yoshida T, Toda Y, Yamada T, Kinukawa N, Terada N, Kobayashi T, Kinoshita H, Kamoto T, Nakamura E, Ogawa O. Increased Akt and phosphorylated Akt expression are associated with malignant biological features of prostate cance in Japanese men. BJU Int 2007;100:685–90. [PubMed: 17542985]

371. Ghosh PM, Malik S, Bedolla R, Kreisberg JI. Akt in prostate cancer: possible role in androgen-independence. Curr Drug Metab 2003;4:487–96. [PubMed: 14683476]

372. Lee JT Jr, McCubrey JA. Targeting the Raf kinase cascade in cancer therapy—novel molecular targets and therapeutic strategies. Expert Opin Ther Targets 2002;6:659–78. [PubMed: 12472379]

373. Malik SN, Brattain M, Ghosh PM, Troyer DA, Prihoda T, Bedolla R, Kreisberg JI. Immunohistochemical demonstration of phospho-Akt in high Gleason grade prostate cancer. Clin Cancer Res 2002;8:1168–71. [PubMed: 11948129]

374. Yeh PY, Chuang SE, Yeh KH, Song YC, Chang LL, Cheng AL. Phosphorylation of p53 on Thr55 by ERK2 is necessary for doxorubicin-induced p53 activation and cell death. Oncogene 2004;23:3580–8. [PubMed: 15116093]

375. Schweyer S, Soruri A, Meschter O, Heintze A, Zschunke F, Miosge N, Thelen P, Schlott T, Radzun HJ, Fayyazi A. Cisplatin-induced apoptosis in human malignant testicular germ cell lines depends on MEK/ERK activation. Br J Cancer 2004;91:589–98. [PubMed: 15266324]

376. Xiao D, Singh SV. Phenethyl isothiocyanate-induced apoptosis in p53-deficient PC-3 human prostate cancer cell line is mediated by extracellular signal-regulated kinases. Cancer Res 2002;62:3615–9. [PubMed: 12097262]

377. van Brussel JP, Mickisch GH. Multidrug resistance in prostate cancer. Onkologie 2003;26:175–81. [PubMed: 12771527]

378. van Brussel JP, van Steenbrugge GJ, Romijn JC, Schroder FH, Mickisch GH. Chemosensitivity of prostate cancer cell lines and expression of multidrug resistance-related proteins. Eur J Cancer 1999;35:664–71. [PubMed: 10492644]

379. Wendel HG, Lowe SW. Reversing Drug Resistance In Vivo. Cell Cycle 2004;3:847–9. [PubMed: 15190216]

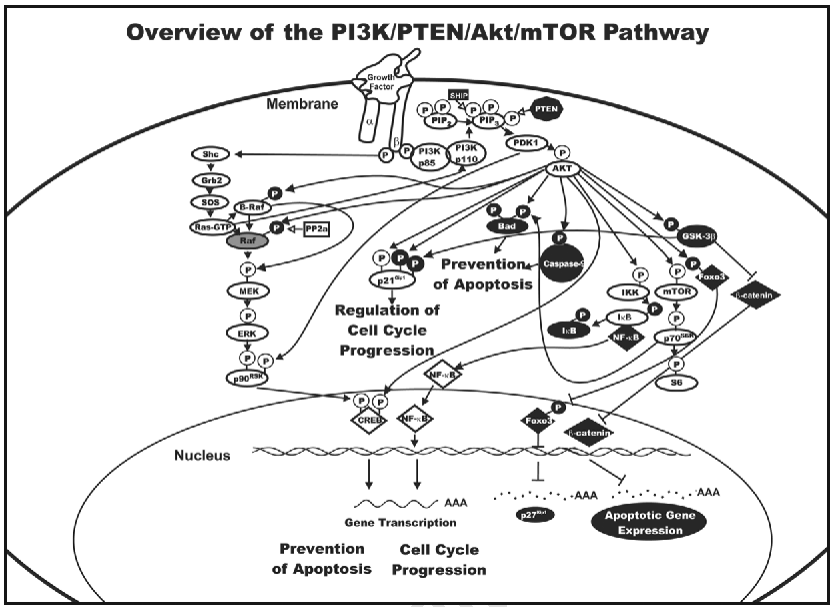

Figure 1.

Overview of PI3K/PTEN/Akt/mTOR Pathway. The PI3K/PTEN/Akt/mTOR pathway is regulated by Ras as well as various kinases. The PI3K/PTEN/Akt/mTOR pathway is also activated after receptor ligation. The PTEN phosphatase (black octagon) inhibits activation of PI3K. Downstream of PI3K, Akt has many downstream targets that regulate cell growth and apoptosis. The transcription factors regulated by these pathways are indicated in diamond-shaped outlines. Dotted lines in front of AAA indicate that there is suppression of expression of some genes due to Akt phosphorylation of transcription factors such as Foxo3. Some of the interactions between the PI3K/PTEN/Akt/mTOR and Raf/MEK/ERK pathways are also indicated.

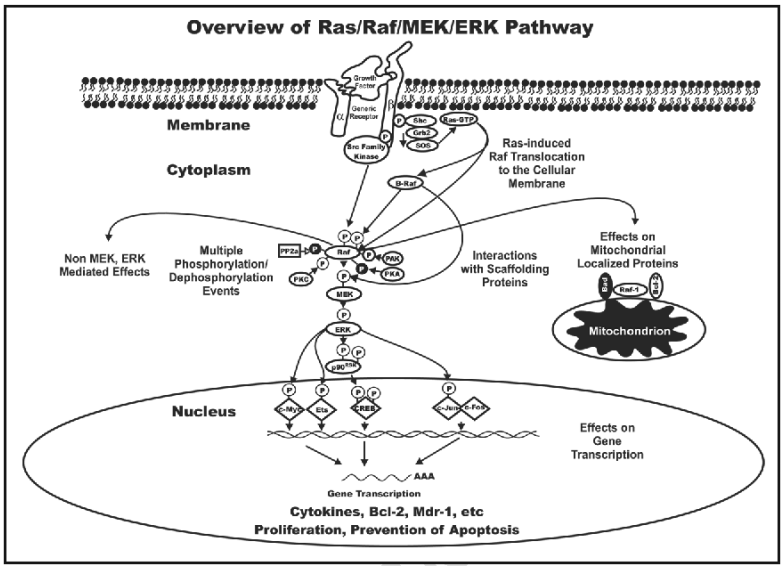

Figure 2.

Overview of Raf/MEK/ERK Pathway. The Raf/MEK/ERK pathway is regulated by Ras as well as various kinases (PKC, PAK, PKA). Many kinases serve to phosphorylate S/T and Y residues on Raf. Some of these phosphorylation events serve to enhance Raf activity (black P in a white circle) whereas others serve to inhibit Raf activity (white P in a black circle. Moreover there are phosphatases such as PP2A, which remove phosphates on certain regulatory residues. The downstream transcription factors regulated by this pathway are indicated in diamond-shaped outlines. Raf can also exert effects which are independent of MEK/ERK and can interact with mitochondrial proteins to regulate apoptosis.

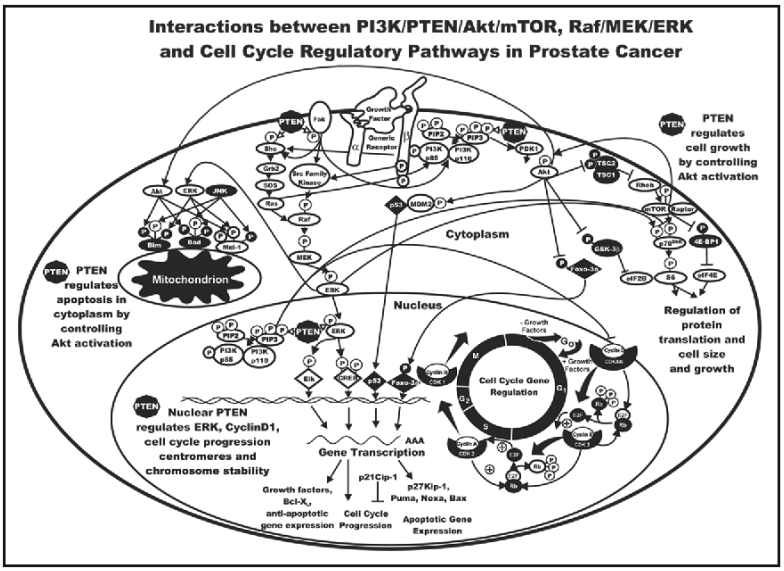

Figure 3.

Interactions Between PI3K/PTEN/Akt/mTOR, Raf/MEK/ERK and Cell Cycle Pathways in Prostate Cancer. All of these pathways interact to regulate the induction of cell cycle progression and apoptosis. The Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways normally serve to suppress apoptosis while p53, which is induced by certain chemotherapeutic drugs and ionizing radiation, will result in increases in pro-apoptotic family members and in some cases, growth factors which may activate certain growth factor receptors. p53 can also regulate the transcription of many genes including $p21^{Cip\text{-}1}$, Bax, Puma, Noxa and other genes which serve to regulate cell cycle progression and the induction of apoptosis. Furthermore, p53 activity can be altered by phosphorylation by ERK as well as MDM2 levels, whose activity is in turn previously regulated by Akt. Hence these pathways are interconnected and serve to regulate each other.

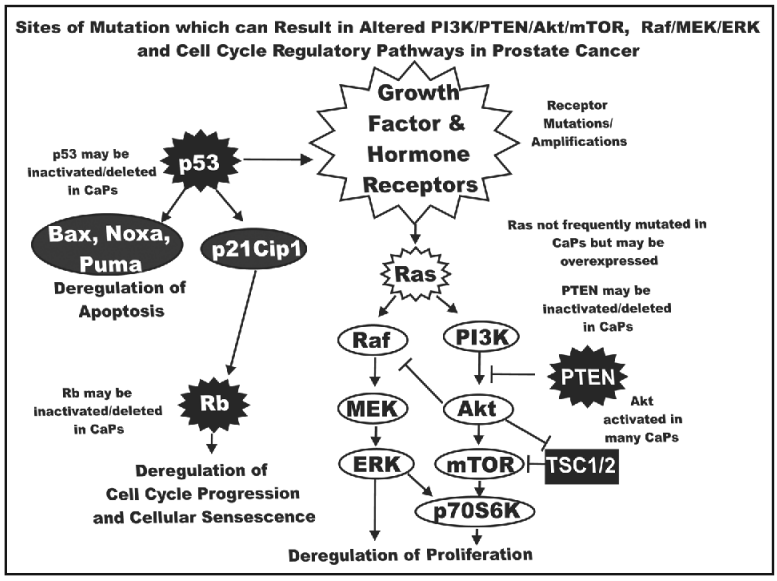

Figure 4.

Sites of Mutation which can Result in Altered PI3K/PTEN/Akt/mTOR Raf/MEK/ERK and Cell Cycle Pathways in Prostate Cells. Mutations and deletions have been detected in p53, PTEN, Rb, AR and many other genes in prostate cancer. Many of these mutations and chromosomal trans-locations result in activation or inactivation of the Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR cascades as well as pathways which control cell cycle progression and apoptosis. The most frequently mutated genes are indicated by a starburst symbol.

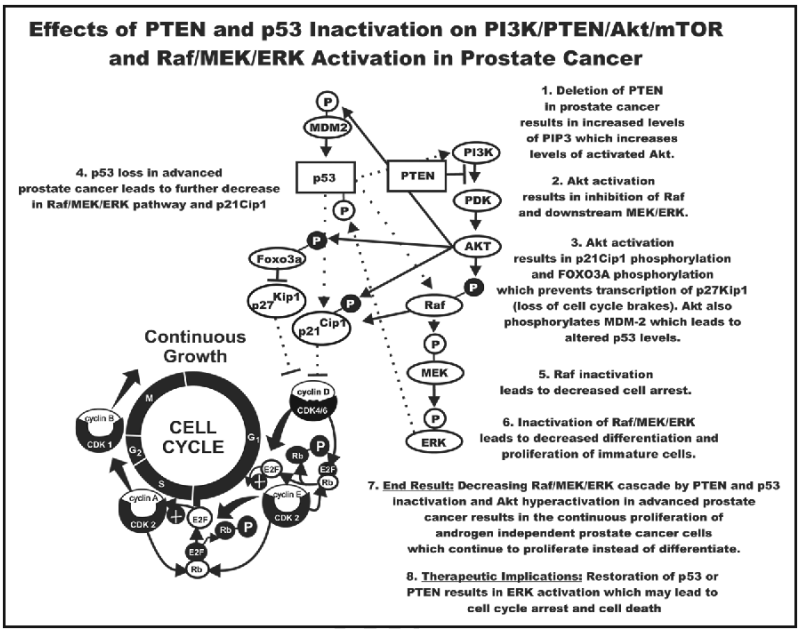

Figure 5.

Effects of PTEN and p53 Inactivation on PI3K/PTEN/Akt/mTOR and Raf/MEK/ERK Activation in Prostate Cancer. Some of the complex interactions between the PI3K/PTEN/Akt/mTOR, Raf/MEK/ERK, p53 and cell cycle pathways and how they influence cell cycle progression in prostate cancer are presented.