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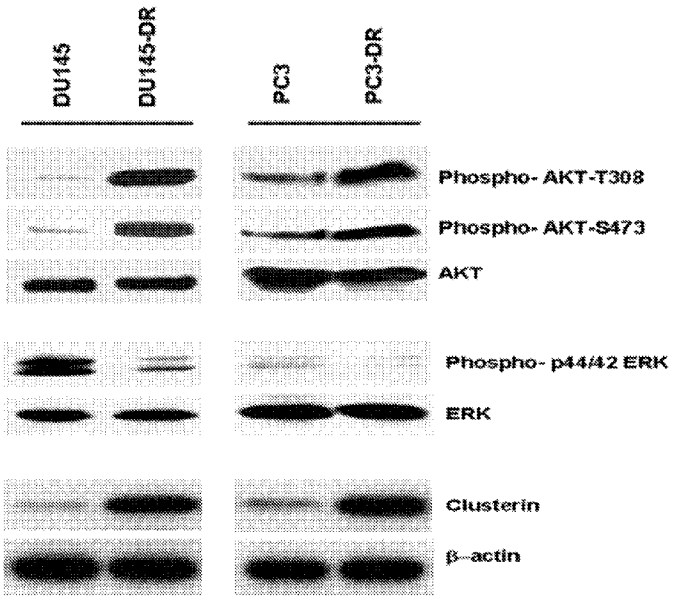
(54) Title: AKT AND CLUSTERIN AS BIOMARKERS OF CHEMOTHERAPEUTIC RESPONSIVENESS

(57) Abstract: Compositions and methods for identifying cancers resistant to treatment due to the presence of elevated pAKT levels and/or overexpression of AKT. The methods comprise the detection of active phosphorylated (p)AKT in a cell or sample of cells. The methods can further include treating the patient with one or more inhibitors of AKT or clusterin responsive to the detection of binding of the antibody to the activated AKT protein or detection of overexpression of AKT. It is found that treatment with AKT or clusterin inhibitors resensitizes advanced cancers to treatment with chemotherapeutics such as docetaxel.

Figure 1



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AKT AND CLUSTERIN AS BIOMARKERS OF CHEMOTHERAPEUTIC RESPONSIVENESS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to currently pending U.S. Provisional Patent Application 61/096,442, entitled, "AKT and Clusterin as Biomarkers of Responsiveness to Chemotherapy in Prostate Cancer", filed September 12, 2008, the contents of which are herein incorporated by reference.

FIELD OF INVENTION

This invention relates to methods and compositions for the diagnosis and treatment of prostate cancer.

BACKGROUND OF THE INVENTION

Prostate cancer is the most common malignancy in men. The current standard of care for hormone-refractory prostate cancer is treatment with docetaxel. A complete cure is often impaired by resistance of the cell to docetaxel. Understanding the molecular basis for docetaxel resistance is pivotal for the identification of new therapeutic targets to achieve cure.

One of the most prominent changes associated with advanced tumor development and taxane resistance in prostate cancer is clusterin (CLU) overexpression [Shannan B, et al. *Cell Death Differ* (2006) Jan;13(1):12-19]. Recent data has shown that the CLU gene gives rise to at least two protein forms; a secreted heterodimeric isoform, sCLU, and an alternatively spliced isoform that mainly localizes in the nucleus, nCLU. Although mature sCLU is processed through the ER-golgi secretory pathway, new evidence suggests that it may evade secretion and localize to the mitochondria and cytosol as a cytoplasmic product [Nizard P, et al. *Traffic* (2007) May;8(5):554-65]. It is this cytoplasmic sCLU that is consistently reported to be associated with chemoresistance and it is present in a wide range of advanced cancers as demonstrated in human tumor biopsies from prostate [Steinberg J, et al. *Clin Cancer Res* (1997) Oct;3(10):1707-11], renal, breast, ovarian, colon, lung, pancreas, and cervical cancer, melanoma, gliomas, and anaplastic large cell lymphoma.

Experimentally, sCLU overexpression in androgen-dependent prostate cancer cells has been shown to render them resistant to androgen-starvation, radiation and paclitaxel treatment [Miyake H, et al. *Cancer Res* (2000) May 1;60(9):2547-54; Miyake H, et al. *Cancer Res* (2000) Jan 1;60(1):170-6; Zellweger T, et al. *Clin Cancer Res* (2002) Oct;8(10):3276-84]. Moreover, anti-sense oligonucleotides or siRNA specific for clusterin can resensitize resistant

prostate tumor cells to docetaxel [Sowery RD, et al. *BJU Int* (2008) 102:389-397]. Thus, sCLU can protect against both taxane compounds, paclitaxel and docetaxel. Interestingly, sCLU also controls resistance to TRAIL [Sallman DA, et al. *Mol Cancer Ther* (2007) Nov;6(11):2938-47] and other chemotherapeutic agents such as doxorubicin, camphothecin, cisplatin, 5-fluorouracil, dacarbazine and etoposide, thus suggesting its central role in drug resistance [Hoeller C, et al. *J Invest Dermatol* (2005) Jun;124(6):1300-7; Lourda M, et al. *J. Int J Cancer* (2007) Feb 1;120(3):611-22; Miyake H, et al. *Clin Cancer Res* (2001) 12:4245-52]. However, the molecular processes involved in CLU expression in tumor cells has remained unclear.

Some studies have focused on CLU gene induction and its promoter region. These studies have revealed the participation of several transcription regulators including Egr-1, API, Heat Shock Factor1/2, b-MYB, and c-MYC. In addition, it appears that STAT1, but not STAT3, is required for CLU gene expression in tumor cells. However, the signal processes upstream of transcription factors involved in CLU induction are not well delineated. AKT has been reported to be activated in advancing prostate cancer [Edwards J, et al. *Clin Cancer Res* (2003) 14:5271-81]. Additionally, there have been unrelated reports that CLU correlates with docetaxel resistance in prostate cancer. However, no link has been previously explored or established between AKT and sCLU gene expression, nor has there been an exploration or correlation between this pathway and docetaxel resistance in prostate tumor cells. Additionally, we have previously reported the STAT1 requirement for CLU induction [Patterson SG, et al. *Oncogene* (2006) 45:6113-22]. However, the ability of AKT to influence STAT1 function has not been previously established.

Accordingly, there remains a long-felt, but unmet need for additional biomarkers predictive of response to chemotherapeutics in prostate, renal, breast, ovarian, colon, lung, pancreas, and cervical cancer, melanoma, gliomas, and anaplastic large cell lymphoma where the responsiveness, or lack thereof, is associated with CLU expression, particularly sCLU expression. Additionally, there remains an important need for additional treatment regimens and therapeutics to overcome the unresponsiveness of cells, such as in prostate cancer, to treatment with traditional therapies including taxanes. The present invention meets these important needs, and others, as will become apparent in the teachings that follow.

SUMMARY OF INVENTION

Compositions and methods for identifying cancers resistant to treatment due to the presence of elevated pAKT levels and/or overexpression of AKT. The methods comprise the detection of active phosphorylated (p)AKT in a cell or sample of cells. The methods can further include treating the patient with one or more inhibitors of AKT or clusterin responsive to the detection of binding of the antibody to the activated AKT protein or detection of overexpression of AKT.

It is found that treatment with AKT or clusterin inhibitors resensitizes advanced cancers to treatment with chemotherapeutics such as docetaxel.

In a first aspect there is provided a method of treating prostate cancer in a patient. The method includes the steps of obtaining a sample of the prostate cancer cell population from the patient, contacting the sample with at least one antibody, wherein the antibody specifically binds to an activated AKT, detecting binding of the antibody to the activated AKT protein, wherein binding is indicative of resistance to treatment of the cancer cell with one or more taxane drugs, pretreating the patient with one or more inhibitors of AKT or sCLU responsive to the detection of binding of the antibody to the activated AKT protein, and treating the patient with a taxane drug or mitoxantrone.

The method can further include the step of screening the sample for overexpression of sCLU. Overexpression is indicative of resistance to treatment of the cancer cell with taxane drug or mitoxantrone. In certain embodiments the overexpression is detected by binding of antibody specific to sCLU. Alternatively, overexpression of sCLU can be detected using nucleic acids complementary to the CLU nucleic acid or to the sCLU nucleic acid. The one or more inhibitors of sCLU can be antisense oligonucleotides or siRNA. The taxane drug can be docetaxel or paclitaxel. AKT inhibitors useful for the inhibition of AKT include API-2 and perifosine. Similarly, the one or more inhibitors of sCLU can be an oligonucleotide that targets clusterin and that has a sequence complementary to clusterin-encoding mRNA.

In a second aspect there is provided a method of chemosensitizing a prostate cancer cell population. The method includes the steps of obtaining a sample of the prostate cancer cell population, contacting the sample with at least one antibody, wherein the antibody specifically binds to an activated AKT, detecting binding of the antibody to the activated AKT protein, wherein binding is indicative of resistance to treatment of the cancer cell with one or more chemotheraputics and contacting the prostate cancer cell population with one or more inhibitors of sCLU or STAT1 responsive to the detection of binding of the antibody to the activated AKT protein. The one or more inhibitors of sCLU can be antisense oligonucleotides or siRNA.

The method can further include the step of contacting the cancer cell population with a taxane drug. The taxane drug can be docetaxel or paclitaxel. AKT inhibitors useful for the inhibition of AKT include API-2 and perifosine. Similarly, the one or more inhibitors of sCLU can be an oligonucleotide that targets clusterin and that has a sequence complementary to clusterin-encoding mRNA.

Alternatively, or in conjunction with taxane treatment, the method can further include the step of contacting the cancer cell population with a chemotherapeutic selected from the group

consisting of doxorubicin, camphothecin, cisplatin, 5-fluorouracil, dacarbazine, etoposide, mitoxantrone and TRAIL.

In still further embodiments the method can include the step of screening the sample for overexpression of sCLU, wherein overexpression is further indication of resistance to treatment of the cancer cell with one or more chemotheraputics. In certain embodiments thereof the overexpression is detected by binding of antibody specific to sCLU. Alternatively, screening for overexpression detects overexpressed sCLU using nucleic acids complementary to the CLU or sCLU nucleic acid.

In a third aspect there is provided a second method of chemosensitizing a prostate cancer cell population. The method includes the steps of obtaining a sample of the prostate cancer cell population, screening the cancer cell population for overexpression of AKT, wherein overexpression is indicative of resistance to treatment of the cancer cell with one or more chemotheraputics and contacting the cancer cell population with one or more inhibitors of sCLU or STAT1 responsive to the detection of overexpression of AKT. The step of screening for overexpression can detect overexpressed AKT using nucleic acids complementary to the AKT nucleic acid. Alternatively, the step of screening for overexpression can detect overexpressed AKT using antibodies specific for AKT or pAKT. Inhibitors of sCLU include antisense oligonucleotides and siRNA.

The method can further include the step of contacting the cancer cell population with a taxane drug or mitoxantrone. Taxane drugs include docetaxel and paclitaxel. Alternatively, or in conjunction with taxane treatment, the method can further include the step of contacting the cancer cell population with a chemotherapeutic selected from the group consisting of doxorubicin, camphothecin, cisplatin, 5-fluorouracil, dacarbazine, etoposide, mitoxantrone and TRAIL. Inhibitors of sCLU include oligonucleotides that target clusterin and that have a sequence complementary to clusterin-encoding mRNA.

In still further embodiments the method can include the step of screening the sample for overexpression of sCLU, wherein overexpression is further indication of resistance to treatment of the cancer cell with one or more chemotheraputics. In certain embodiments thereof the overexpression is detected by binding of antibody specific to sCLU. Alternatively, screening for overexpression detects overexpressed sCLU using nucleic acids complementary to the CLU or sCLU nucleic acid.

In a fourth aspect there is provided a method of evaluating susceptibility to treatment of prostate cancer with a taxane drug or mitoxantrone in a patient. The method includes the steps of obtaining a sample from the patient, contacting the sample with at least one antibody, wherein the antibody specifically binds to an activated AKT, and detecting binding of the

antibody to the activated AKT protein, wherein binding is indicative of resistance to treatment of the prostate cancer.

The method can further include the step of administering two or more treatments to the patient responsive to the detection of binding of the antibody to the activated AKT protein. One of the two or more treatments can be a drug that selectively targets AKT or clusterin. AKT inhibitors include API-2 and perifosine. Similarly, clusterin inhibitors can be antisense oligonucleotides or siRNA. A second of the two or more treatments can be a taxane drug or mitoxantrone. Taxane drug include docetaxel and paclitaxel.

In still further embodiments the method can include the step of screening the sample for overexpression of sCLU, wherein overexpression is indicative of resistance to treatment of the cancer cell with one or more chemotherapeutics. Overexpression can be detected by binding of antibody specific to sCLU. Alternatively, screening for overexpression can detect overexpressed sCLU using nucleic acids complementary to the CLU or sCLU nucleic acid.

In a fifth aspect there is provided a method of evaluating susceptibility to treatment of prostate cancer with a taxane drug or mitoxantrone in a patient. The method includes the steps of obtaining a sample from the patient and screening the cancer cell population for overexpression of AKT, wherein overexpression is indicative of resistance to treatment of the cancer cell with a taxane drug or mitoxantrone.

In a sixth aspect there is provided a method of inducing apoptosis in a docetaxel resistant prostate cancer cell. The method includes the steps of pretreating the cell with an AKT inhibitor and treating the cell with docetaxel. The AKT inhibitor can be API-2 or perifosine.

In a seventh aspect there is provided a second method of treating prostate cancer in a patient. The method can include the steps of obtaining a sample of the prostate cancer cell population from the patient, contacting the sample with at least one antibody, wherein the antibody specifically binds to sCLU, detecting binding of the antibody to the sCLU protein, wherein binding is indicative of resistance to treatment of the cancer cell with one or more taxane drugs, pretreating the patient with one or more inhibitors of AKT or sCLU responsive to the detection of binding of the antibody to the sCLU protein and treating the patient with a taxane drug or mitoxantrone.

The method can further include the step of screening the sample for overexpression of AKT, wherein overexpression is further indication of resistance to treatment of the cancer cell with taxane drug or mitoxantrone. Overexpression can detected by binding of antibody specific to pAKT or AKT. Alternatively, screening for overexpression can detect overexpressed AKT using nucleic acids complementary to the AKT nucleic acid. Inhibitors of sCLU include antisense oligonucleotides and siRNA. Taxane drugs include docetaxel and paclitaxel. AKT

inhibitors include API-2 and perifosine. The inhibitors of sCLU can be an oligonucleotide that targets clusterin and that has a sequence complementary to clusterin-encoding mRNA.

In an eighth aspect there is provided a third method of chemosensitizing a prostate cancer cell population. The method includes the steps of obtaining a sample of the prostate cancer cell population, contacting the sample with at least one antibody, wherein the antibody specifically binds to sCLU, detecting binding of the antibody to the sCLU protein, wherein binding is indicative of resistance to treatment of the cancer cell with one or more chemotheraputics and contacting the prostate cancer cell population with one or more inhibitors of sCLU or STAT1 responsive to the detection of binding of the antibody to the sCLU protein. Inhibitors of sCLU include antisense oligonucleotides and siRNA. The inhibitors of sCLU can be an oligonucleotide that targets clusterin and that has a sequence complementary to clusterin-encoding mRNA.

The method can further include the step of contacting the cancer cell population with a taxane drug or mitoxantrone. Taxane drugs include docetaxel and paclitaxel. Alternatively, or in conjunction with taxane treatment, the method can further include the step of contacting the cancer cell population with a chemotherapeutic selected from the group consisting of doxorubicin, camphothecin, cisplatin, 5-fluorouracil, dacarbazine, etoposide, mitoxantrone and TRAIL. Inhibitors of sCLU include oligonucleotides that target clusterin and that have a sequence complementary to clusterin-encoding mRNA.

The method can further include the step of screening the sample for overexpression of AKT, wherein overexpression is further indication of resistance to treatment of the cancer cell with one or more chemotheraputics. Overexpression can detected by binding of antibody specific to pAKT or AKT. Alternatively, screening for overexpression can detect overexpressed AKT using nucleic acids complementary to the AKT nucleic acid. Inhibitors of sCLU include antisense oligonucleotides and siRNA. Taxane drugs include docetaxel and paclitaxel. AKT inhibitors include API-2 and perifosine. The inhibitors of sCLU can be an oligonucleotide that targets clusterin and that has a sequence complementary to clusterin-encoding mRNA.

In a ninth aspect there is provided a fourth method of chemosensitizing a prostate cancer cell population. The method includes the steps of obtaining a sample of the prostate cancer cell population, screening the cancer cell population for overexpression of sCLU, wherein overexpression is indicative of resistance to treatment of the cancer cell with one or more chemotherapeutics, and contacting the cancer cell population with one or more inhibitors of sCLU or STAT1 responsive to the detection of overexpression of sCLU. The step of screening for overexpression can detect overexpressed sCLU using nucleic acids complementary to the sCLU nucleic acid. Alternatively, the step of screening for overexpression can detect overexpressed sCLU using antibodies specific for sCLU. The method according to claim 64

further comprising the step of contacting the cancer cell population with a taxane drug or mitoxantrone.

The method can further include the step of screening the sample for overexpression of AKT, wherein overexpression is indicative of resistance to treatment of the cancer cell with one or more chemotheraputics. Overexpression can detected by binding of antibody specific to pAKT or AKT. Alternatively, screening for overexpression can detect overexpressed AKT using nucleic acids complementary to the AKT nucleic acid. Inhibitors of sCLU include antisense oligonucleotides and siRNA. Taxane drugs include docetaxel and paclitaxel. AKT inhibitors include API-2 and perifosine. The inhibitors of sCLU can be an oligonucleotide that targets clusterin and that has a sequence complementary to clusterin-encoding mRNA.

In a tenth aspect there is provided a second method of evaluating susceptibility to treatment of prostate cancer with a taxane drug or mitoxantrone in a patient. The method includes the steps of obtaining a sample from the patient, contacting the sample with at least one antibody, wherein the antibody specifically binds to sCLU, and detecting binding of the antibody to the sCLU protein, wherein binding is indicative of resistance to treatment of the prostate cancer.

The method can further include the step of administering two or more treatments to the patient responsive to the detection of binding of the antibody to the sCLU protein. One of the two or more treatments can be a drug that selectively targets AKT or clusterin. AKT inhibitor can include API-2 and perifosine. Clusterin inhibitors include antisense oligonucleotides and siRNA. The method can further include the step of screening the sample for overexpression of AKT, wherein overexpression is indicative of resistance to treatment of the cancer cell with one or more chemotheraputics. Overexpression can detected by binding of antibody specific to pAKT or AKT. Alternatively, screening for overexpression can detect overexpressed AKT using nucleic acids complementary to the AKT nucleic acid. Inhibitors of sCLU include antisense oligonucleotides and siRNA. Taxane drugs include docetaxel and paclitaxel. AKT inhibitors include API-2 and perifosine. The inhibitors of sCLU can be an oligonucleotide that targets clusterin and that has a sequence complementary to clusterin-encoding mRNA.

In a eleventh aspect there is provided a fifth method of chemosensitizing a prostate cancer cell population. The method includes the steps of obtaining a sample of the prostate cancer cell population, wherein the cancer is a cancer characterized by elevated sCLU, contacting the sample with at least one antibody, wherein the antibody specifically binds to an activated AKT, detecting binding of the antibody to the activated AKT protein, wherein binding is indicative of resistance to treatment of the cancer cell with one or more chemotherapeutics, and contacting the cancer cell population with one or more inhibitors of sCLU or STAT1 responsive to the detection of binding of the antibody to the activated AKT protein.

BRIEF DESCRIPTION OF THE DRAWINGS

For a fuller understanding of the invention, reference should be made to the following detailed description, taken in connection with the accompanying drawings, in which:

FIG. 1 shows that AKT activation accompanies docetaxel-resistance in DU145 and PC3 human prostate tumor cells. Paired parental and docetaxel-resistant (DR) DU145 and PC3 prostate tumor cells were lysed and analyzed by western blotting with specific antibodies against CLU. Activated AKT was evaluated using antibodies directed against the phosphorylation site at Thr 308 or Ser 473, while anti-pERK detected both p42 ERK1 and p44 ERK2 isoforms. Equal loading was monitored by β-actin levels. Immunoblot analysis of phospho-AKT, phospho-ERK and sCLU indicated that active AKT and sCLU were increased in DU145-DR and PC-DR cells, as compared to their matched parental cells, while active ERK showed the opposite trend.

FIG. 2 shows that the API-2 blockade of AKT function reduces sCLU expression and induces chemosensitivity to docetaxel in drug-resistant tumor cells. In FIG. 2A DU145-DR and PC3-DR tumor cells were pretreated 2 h with the indicated doses of API-2, a potent AKT inhibitor, and exposed to docetaxel-containing medium for 48 h. Cell lysates were then prepared and analyzed by western blotting with antibodies against phospho-AKT, phospho-ERK. The lysates were also probed with antibodies specific for total AKT, total ERK and sCLU. Treatment with API-2 caused a dose-dependent inhibition of active AKT and sCLU protein expression but an increase in active ERK. In FIG. 2B DU145-DR and PC3-DR tumor cells, similarly treated with API-2, were analyzed for cell apoptosis by Annexin V/PI staining, after a further 48 h culture in docetaxel medium. Both cell lines succumbed to docetaxel-induced cell death after API-2 treatment to deplete AKT function.

FIG. 3 shows that the expression of dominant-negative (DN)-AKT disrupts sCLU expression. In FIG. 3A DU145-DR and PC3-DR tumor cells were transfected with HA-tagged DN-AKT and cultured for 48 h in docetaxel-containing medium. Control transfection was with the empty vector, PCDNA3. Transfection efficiency was monitored by western blot analysis with anti-HA and anti-AKT, and its function was assessed by analysis of GSK-3β phosphorylation, which is a known AKT target. DN-AKT effectively suppressed GSK-3β phosphorylation and correspondingly suppressed sCLU expression. For specificity control, ERK phosphorylation was evaluated and DN-AKT was found not to suppress ERK but instead enhanced its phosphorylation. In FIG. 3B analysis of sCLU gene expression was performed by RT-PCR on DU145Dr and PC3-DR cells that were either treated with DMSO or API-2, or transfected with control PCDNA3 vector or DN-AKT. Both API-2 treatment and DN-AKT transfection markedly suppressed clusterin mRNA expression in DU145-DR and PC3-DR tumor cells, as compared to their respective controls. Equal loading was monitored by GAPDH mRNA levels.

FIG. 4 shows that AKT inhibition by API-2 treatment or DN-AKT transfection induces chemosensitivity to docetaxel via a caspase-3 dependent pathway. In FIG. 4A DU145-DR and PC3-DR tumor cells, pretreated with API-2 or DN-AKT transfection (including DMSO or PCDNA3 controls) were treated with zVAD.fmk, a general caspase inhibitor, and zVAD.amc, a caspase-1-specific inhibitor. These cells were then exposed to docetaxel for 48 h prior to analysis of active caspase-3. High levels of active caspase-3 were detected in API-treated and DN-AKT expressing tumor cells. This activity was inhibited by zVAD.fmk but not zVAD.amc. In FIG. 4B visual assessment of cell survival by methylene blue staining of the same cells show few cells surviving in DU145DR and PC3-DR tumor cells treated with API-2 or transfected with DN-AKT, while the control DMSO-treated and PCDNA-3 expressing cells showed a robust growth.

FIG. 5 shows that overexpression of sCLU overcomes API-2-induced chemosensitivity to docetaxel. In FIG. 5A DU145-DR tumor cells were transfected with either control PCDNA3 or myc-tagged sCLU. These cells were then pretreated with API-2 for 2 h and incubated in docetaxel medium for 48 h. Western blot analysis with anti-myc was performed to confirm efficient transfection and further analysis of sCLU, phospho-AKT and total AKT was pursued. It is confirmed that API-2 can suppress AKT function in both PCDNA3 and sCLU-expressing tumor cells. In FIG. 5B these same cells were also analyzed for cell growth and survival by methylene blue staining. API-2 effectively induced docetaxel sensitivity and cell death in DU145-DR tumor cells but could not do so in the sCLU-overexpressing DU145-DR tumor cells.

FIG. 6 shows that inhibition of AKT function leads to STAT1 inactivation. DU145-DR tumor cells, pretreated with API-2 or DMSO in one set or transfected with DN-AKT or PCDNA3 control vector in a second set, were further cultured 24 h in docetaxel medium. Cells were then lysed and analyzed by western blotting with anti-phospho-STAT1 or total STAT1. Equal loading was also monitored with anti-β-actin. P-STAT1 levels in DU145 DR cells either transfected with DNAKT or treated with API-2. Transfected cells were harvested 48hrs post transfection. API-2 treated cells were harvested after 24 hrs.

FIG. 7 shows immunohistochemical analysis of 45 prostate cancer patient tumor specimens. In FIG. 7A representative Gleason 6, Gleason 8+ and metatastic prostate tumor sample is shown and indicates minimal clusterin and pAKT expression in the low grade Gleason 6 tumor, with a rise of both in the Gleason 8+ tumor, progressing to even higher levels in the metastatic tumor. In FIG. 7B an analysis of 12 Gleason 6 tumors, 12 Gleason 7 tumors, 12 Gleason 8+ tumors and 9 metastatic tumors indicated that there is a consistent progression towards higher expression of both clusterin and pAKT levels with higher grade of tumors, that is statistically significant, $p<0.0001$. FIG 7C is a graph illustrating the mean (S.E.) of clusterin

and pAKT levels for each grade of tumors and shows a steady rise in both clusterin and pAKT with advancing Gleason scores.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention provides compositions and methods for identifying cancers resistant to treatment due to the presence of elevated pAKT levels, particularly prostate cancer that is refractive to treatment with taxanes such as docetaxel. The methods comprise the detection of active phosphorylated (p)AKT in a cell or sample of cells. The presence of pAKT is shown below to strongly correlate with the sCLU level in a cell. Moreover, sCLU levels have been shown to confer resistance to various therapeutic, as discussed herein, for the treatment of cancer. Therefore, the biomarkers of the invention are capable of distinguishing samples that are likely to be resistant to treatment with one or more modalities from those which are likely to respond to treatment. Moreover, detection of the pAKT can lead to tailored treatments such as the administration of agents directed against pAKT or other downstream targets, including STAT1 and sCLU. Methods for identifying cancers resistant to treatment due to elevated pAKT levels, and consequently overexpression of sCLU, involve detecting the presence of at least one biomarker that is indicative of elevated pAKT levels in a cell sample. In certain aspects of the invention, the methods permit the tailoring of cancer therapy. In particular embodiments, antibodies and immunochemistry techniques are used to detect expression of the biomarker of interest. Kits for practicing the methods of the invention are further provided.

Clusterin (CLU), in its cytoplasmic form, is abundant in many advanced cancers. It has been established to be cytoprotective against chemotherapeutic agents including docetaxel. However, little is known of the mechanism of its induction. Here we provide evidence that AKT plays a critical role in upregulating cytoplasmic/secretory sCLU which is responsible for docetaxel resistance. Western blot analysis indicates that docetaxel-resistant (DR) sublines derived from DU145 and PC3 prostate tumor cell lines displayed a markedly increased phospho-AKT level closely accompanied by heightened sCLU expression when compared to the parental cells. To examine if AKT has a role in CLU expression, AKT blockade was performed by treatment with a specific inhibitor, API-2, or dominant-negative AKT transduction, prior to analysis of CLU gene expression. Loss of AKT function resulted in loss of sCLU and was accompanied by chemosensitization to docetaxel and increased cell death via a caspase 3-dependent pathway. To confirm that AKT affected resistance to docetaxel through CLU, and not through other mediators, tumor cells were first transfected with CLU for overexpression. The cells then were treated with the AKT inhibitor, API-2. Once CLU was overexpressed, API-2 could not chemosensitize the tumor cells to docetaxel. Thus, the chemoresistance to docetaxel is mediated by CLU, which can be induced by AKT. Lastly, AKT was found to mediate CLU induction via Stat1 activation, which has been shown to drive

sCLU gene expression. These results identify a previously unrecognized pathway linking AKT to cytoprotection by clusterin in tumor cells.

Thus, in aspects of the present invention there is provided insight into the development of docetaxel resistance in prostate cancer. Despite evidence implicating clusterin in paclitaxel/docetaxel resistance [Miyake H, et al. *Cancer Res* (2000) 9:2547-54; Patterson SG, et al. *Oncogene* (2006) 45:6113-22] and reports of AKT activation in prostate tumor cells [Edwards J, et al. *Clin Cancer Res* (2003) 14:5271-81], there has been no link between these two anti-apoptotic proteins. A new pathway is identified herein by which AKT can prevent cell death. It does so by acting as a key positive transcriptional regulator of sCLU. The evidence for this AKT-sCLU pathway was obtained by analysis of biological, biochemical and molecular functions. It is shown below that AKT was highly up-regulated in the docetaxel-resistant cell lines, DU145-DR and PC3-DR, in comparison to their wild type counterparts. Second, AKT inhibition was demonstrated. The demonstration was made using either a pharmacological agent, API-2, or by overexpression of dominant-negative AKT. This inhibition can markedly suppress sCLU gene expression in the drug-resistant cells and re-sensitize these tumor cells to docetaxel. Finally, it was shown that DU145-DR cells could be retained from succumbing to API-2-induced docetaxel sensitivity by overexpressing sCLU. This showing highlights the importance of sCLU expression in docetaxel resistance and as a pro-survival factor.

These findings suggest that other factors, such as bcl-2, survivin or XIAP, are not involved. Indeed, it has been shown that paclitaxel and docetaxel block bcl-2 function. These taxanes are able to block by disrupting the microtubule integrity that leads to bcl-2 phosphorylation and inactivation in tumor cells. This property is the basis for the clinical use of docetaxel in patients with androgen-refractory prostate cancer who acquire abnormally activated bcl-2 in their tumors. In addition to bcl-2, others have investigated the participation of survivin, XIAP and sCLU in camptothecin resistance in prostate tumor cells. In analysis of camptothecin-resistant PC3 tumor cells, treatment with camptothecin was found to effectively reduce survivin and XIAP expression but, on the contrary, it markedly upregulated sCLU expression. Therefore, sCLU may be a common factor raised in tumor cells that develop resistance to chemotherapeutic agents.

Expression of sCLU expression has been shown to be dependent on STAT1 [Patterson SG, et al. *Oncogene* (2006) 45:6113-22], although there has been no linkage previously shown between AKT and STAT1 activation. Confirmation of this linkage is provided below, showing that AKT does activate STAT1. This was done by demonstrating that API2 treatment or DN-AKT expression was capable of suppressing STAT1 activation in DU145-DR tumor cells. These results explain the recent linkage of AKT to paclitaxel and TRAIL resistance [Cheng GZ, et al. *Cancer Res* (2007) 5:1979-87; Nesterov A, et al. *J Biol Chem* (2001) 14:10767-74].

Although AKT was found to mediate such resistance, the survival factor in both cases was unknown. Here, we have definitive data to identify sCLU as the link between AKT and paclitaxel/docetaxel resistance. We also recently demonstrated that sCLU is responsible for TRAIL resistance in prostate tumor cells [Sallman DA, et al. *Mol Cancer Ther* (2007) 11:2938-47]. Thus, an association can now be made and explained, according to the teachings herein, between AKT and TRAIL resistance. It has also been shown that a tyrosine-kinase inhibitor, resveratrol, was able to restore TRAIL sensitivity in resistant prostate tumor cells, adding the possibility of a Src kinase in the upregulation of sCLU. One possible explanation is that Src activation leads to AKT activation resulting in STAT1 upregulation of the CLU gene.

It is shown below that ERK becomes activated upon suppression of AKT in DU145-DR and PC3-DR tumor cells. Either API-2 treatment or DN-AKT transfection causes a marked rise in active ERK. DU145 tumor cells also show a heightened ERK function upon PI3K inhibition [Lee JT, et al. *Cell Cycle* (2008) 5:631-6]. ERK activation has been reported to be essential for drug-induced apoptosis in prostate carcinoma cells. Thus, AKT inhibition appears to allow for ERK activation to mediate cell death.

Emerging evidence, including data from this current report, indicates that in instances of cellular stress, there is an increase in the expression of the ~60 kDa full-length unprocessed sCLU form. This ~60 kDa sCLU has been reported to localize to the cytosol through an unknown mechanism by which it evades the ER-Golgi secretory pathway [Nizard P, et al. *Traffic* (2007) 5:554-65]. Once cytoplasmic, there is evidence that sCLU functions in a prosurvival role during cell death and confer resistance to cytotoxic agents [Patterson SG, et al. *Oncogene* (2006) 45:6113-22; Lourda M, et al. *J. Int J Cancer* (2007) 3:611-22; Trougakos IP, et al. *Cancer Res* (2004) 5:1834-42]. Expression of sCLU is likely to lead to multi-drug resistance as it has been shown to confer protection against a number of clinically-established chemotherapeutic agents, including cisplatin, doxorubicin, camphothecin, dacarbazine, etoposide and 5- fluorouracil [Hoeller C, et al. *J Invest Dermatol* (2005) 6:1300-7; Lee CH, et al. *Urology* (2002) 3:516-20; Lourda M, et al. *J. Int J Cancer* (2007) 3:611-22; Miyake H, et al. *Clin Cancer Res* (2001) 12:4245-52]. In addition to these commonly used anti-cancer drugs, sCLU is a potent disruptor of targeted therapy involving the Tumor Necrosis Factor (TNF) family of proteins, including TNF, Fas, and TRAIL [Sallman DA, et al. *Mol Cancer Ther* (2007) 11:2938-47]. One explanation is that, within the cell, sCLU exerts its cytoprotective effect by binding partially unfolded proteins to prevent stress-induced protein aggregation, and its binding and stabilization of the Ku70-Bax complex is a key factor preventing mitochondria-mediated apoptosis. Such sCLU binding prevents the release of Bax to the mitochondria to initiate cytochrome c and the resultant caspase 3-dependent apoptotic pathway. It is to be noted that, as shown in the examples below, the death of DU145-DR,

upon loss of sCLU, is mediated by caspase 3-induced cell death, confirming that sCLU is utilizing the same pathway in the cells.

In contrast, the nuclear form of clusterin, which is derived by alternative splicing and containing a nuclear localization sequence, appears to function as a pro-death protein in human cancer cells [Yang CR, et al. *Proc Natl Acad Sci U S A* (2000) 11:5907-12]. Its mechanism of action is also via stabilization of the Ku70/Bax complex. However, in the nucleus, Ku70 serves as a critical nuclear factor involved in DNA repair and it is the sequestration of Ku70 by nCLU that impairs repair of DNA damage, thus leading to apoptosis. Only the sCLU form of clusterin was detected in the cell lines used below, which is consistent with other groups also unable to detect the nuclear form in prostate cancer cell lines.

The discovery of the AKT-clusterin axis reported herein impacts numerous facets of the current protocols for the treatment of cancer, including prostate cancer. Acquisition of high pAKT levels, often seen in advanced cancer, are associated with increased sCLU expression and become a barrier to successful docetaxel therapy for these patients. Positive screening of these molecular markers could be used to divert treatment towards drugs that selectively target AKT and/or clusterin. AKT inhibitors such as perifosine, API-2 [Yang L, et al. *Cancer Res* (2004) 13:4394-9], and anti-sense oligos targeting clusterin are already in early phase clinical trials [Chi KN, et al. *Clin Cancer Res* (2008) 3:833-9] and they could improve cancer sensitivity in combination with docetaxel, or other anti-tumor therapeutics. This strategy would also benefit other cancers, including breast, ovarian, colon, lung, renal, pancreas, cervical and bladder carcinomas, melanoma, gliomas, which are often treated with docetaxel and other anticancer drugs and express high-levels of clusterin in late-stage tumors.

As used throughout the entire application, the terms "a" and "an" are used in the sense that they mean "at least one", "at least a first", "one or more" or "a plurality" of the referenced components or steps, unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

The term "and/or" whereever used herein includes the meaning of "and", "or" and "all or any other combination of the elements connected by said term".

The term "about" or "approximately" as used herein means within 20%, preferably within 10%, and more preferably within 5% of a given value or range.

Other than in the operating examples, or unless otherwise expressly specified, all of the numerical ranges, amounts, values and percentages such as those for amounts of materials, times and temperatures of reaction, ratios of amounts, values for molecular weight (whether number average molecular weight ("M_n") or weight average molecular weight ("M_w"), and others in the following portion of the specification may be read as if prefaced by the word

"about" even though the term "about" may not expressly appear with the value, amount or range. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present disclosure. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the disclosure are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Furthermore, when numerical ranges of varying scope are set forth herein, it is contemplated that any combination of these values inclusive of the recited values may be used.

As used herein, the term "comprising" is intended to mean that the products, compositions and methods include the referenced components or steps, but not excluding others. "Consisting essentially of" when used to define products, compositions and methods, shall mean excluding other components or steps of any essential significance. Thus, a composition consisting essentially of the recited components would not exclude trace contaminants and pharmaceutically acceptable carriers. "Consisting of" shall mean excluding more than trace elements of other components or steps.

As used herein, the term "pretreating", or "pretreatment", is intended to mean that a first treatment is administered prior to, or in conjunction with, a second treatment. In other words, the pretreatment may be performed before another, later treatment, thus allowing the pretreatment time to take effect. Alternatively, the pretreatment may be performed or administered simultaneously with a second treatment without a temporal delay. Advantageously, a pretreatment is administered prior to a second treatment.

The present invention contemplates the detection of elevated sCLU through the detection of elevated pAKT as well as through the detection of overexpression of AKT. Any methods available in the art for identification or detection of the biomarkers are encompassed herein. The overexpression of a biomarker of the invention can be detected on a nucleic acid level or a protein level. In order to determine overexpression, the sample to be examined may be compared with a corresponding body sample that originates from a healthy person or from a cell population exhibiting levels associated with such healthy state. That is, the "normal" level of expression is the level of expression of the biomarker in a body sample of a human subject or patient not afflicted with the cancer characterized by elevated sCLU. Such a sample can be

present in standardized form. In some embodiments, determination of biomarker overexpression requires no comparison between the sample and a corresponding sample that originates from a healthy state population. In this situation, the biomarker of interest is overexpressed to such an extent that it precludes the need for comparison to a corresponding sample that originates from a healthy state population.

Methods for detecting biomarkers of the invention comprise any methods that determine the quantity or the presence of the biomarkers either at the nucleic acid or protein level. Such methods are well known in the art and include but are not limited to western blots, northern blots, southern blots, enzyme linked immunosorbent assay (ELISA), immunoprecipitation, immunofluorescence, flow cytometry, bead-based immunochemistry, immunochemistry, molecular imprinting, nucleic acid aptamers, nucleic acid hybridization techniques, nucleic acid reverse transcription methods, and nucleic acid amplification methods. In particular embodiments, overexpression of a biomarker is detected on a protein level using, for example, antibodies that are directed against specific biomarker proteins. These antibodies can be used in various methods such as Western blot, ELISA, or immunoprecipitation techniques.

In one embodiment, antibodies specific for biomarker proteins are utilized to detect the overexpression of a biomarker protein in a body sample. The method comprises obtaining a body sample from a patient, contacting the body sample with at least one antibody directed to a pAKT biomarker, and detecting antibody binding to determine if the biomarker is elevated in the patient sample. As noted below, a more accurate diagnosis may be obtained in some cases by detecting more than one biomarker in a patient sample. Therefore, in particular embodiments, at least two antibodies directed to two distinct biomarkers are used to detect elevated sCLU. Where more than one antibody is used, these antibodies may be added to a single sample sequentially as individual antibody reagents or simultaneously as an antibody cocktail. Alternatively, each individual antibody may be added to a separate aliquot from the same sample, and the resulting data pooled. One of skill in the art will recognize that the immunochemistry methods described herein may be performed manually or in an automated fashion.

In an advantageous immunochemistry method of the invention, a two antibody or "sandwich" ELISA is used to detect biomarker overexpression in a patient sample. Such "sandwich" or "two-site" immunoassays are known in the art. See, for example, Current Protocols in Immunology. *Indirect Antibody Sandwich ELISA to Detect Soluble Antigens,* John Wiley & Sons, 1991. In this aspect of the invention, two antibodies specific to two distinct antigenic sites on a single biomarker are used. By "distinct antigenic site" is intended that the antibodies are specific for different sites on the biomarker protein of interest such that binding of one

antibody does not significantly interfere with binding of the other antibody to the biomarker protein. The first antibody, known as the "capture antibody," is immobilized on or bound to a solid support. For example, a capture antibody directed to a biomarker of interest may be covalently or noncovalently attached to a microtiter plate well, a bead, a cuvette, or other reaction vessel. In a preferred embodiment, the capture antibody is bound to a microtiter plate well. Methods for attaching an antibody to a solid support are known in the art. The body sample, particularly a serum sample, is contacted with the solid support and allowed to complex with the bound capture antibody. Unbound sample is removed, and a second antibody, known as the "detection antibody," is added to the solid matrix. The detection antibody is specific for a distinct antigenic site on the biomarker of interest and is coupled to or labeled with a substance that provides a detectable signal. Such antibody labels are well known in the art and include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Following incubation with the detection antibody, unbound sample is removed, and biomarker expression levels are determined by quantitation of the labeled detection antibody bound to the solid support. One of skill in the art will recognize that the capture and detection antibodies can be contacted with the body sample sequentially, as described above, or simultaneously. Furthermore, the detection antibody can be incubated with the body sample first, prior to contacting the sample with the immobilized capture antibody.

Techniques for detecting antibody binding through the use of a detectable label are well known in the art. For example, antibody binding may be detected through the use of chemical reagents that generate a detectable signal that corresponds to the level of antibody binding and, accordingly, to the level of biomarker protein expression. In some embodiments, the detection antibody is coupled to an enzyme, particularly an enzyme that catalyzes the deposition of a chromogen at the antigen-antibody binding site. Enzymes of particular interest include but are not limited to horseradish peroxidase (HRP) and alkaline phosphatase (AP). Commercial antibody detection systems may also be used to practice the invention.

The above-described immunochemistry methods and formats are intended to be exemplary and are not limiting since, in general, it will be understood that any immunochemistry method or format can be used in the present invention.

In aspects of the invention, methods require the detection of at least one biomarker in a sample for the detection of AKT, 2, 3, 4 or more biomarkers may be used to practice the present invention. It is recognized that detection of more than one biomarker in a sample may be used to identify instances of elevated sCLU, wherein the elevated sCLU is determined in part by elevated pAKT or elevated levels of AKT expression. Therefore, in some embodiments, two or more biomarkers are used, more preferably, two or more

complementary biomarkers. By "complementary" is intended that detection of the combination of biomarkers in a sample result in the successful identification of elevated sCLU in a greater percentage of cases than would be identified if only one of the biomarkers was used. Thus, in some cases, a more accurate determination can be made by using at least two biomarkers. Accordingly, where at least two biomarkers are used, at least two antibodies directed to distinct biomarker proteins can be used to practice the immunochemistry methods disclosed herein. The antibodies may be contacted with the sample simultaneously or concurrently.

In particular embodiments, the diagnostic methods of the invention comprise collecting a body sample from a patient, contacting the sample with at least one antibody specific for a biomarker of interest, and detecting antibody binding. Samples that exhibit elevation or overexpression of a biomarker of the invention, as determined by detection of antibody binding to activated AKT, are deemed positive for activated AKT (pAKT).

By "body sample" is intended any sampling of cells, tissues, or bodily fluids in which expression of a biomarker can be detected. Examples of such body samples include but are not limited to blood, lymph, urine, and biopsies. Body samples may be obtained from a patient by a variety of techniques including, for example, by scraping or swabbing an area or by using a needle to aspirate bodily fluids.

The terms "antibody" and "antibodies" broadly encompass naturally occurring forms of antibodies and recombinant antibodies such as single-chain antibodies, chimeric and humanized antibodies as well as fragments and derivatives of all of the foregoing, which fragments and derivatives have at least an antigenic binding site. Antibody derivatives may comprise a protein or chemical moiety conjugated to the antibody.

The term "antibody" is used in the broadest sense and covers fully assembled antibodies, antibody fragments that can bind antigen (e.g., Fab', F'$(ab)_2$, Fv, single chain antibodies, diabodies), and recombinant peptides comprising the foregoing.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen-binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')2, and Fv fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment. Pepsin treatment yields an F(ab')2 fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

Polyclonal antibodies can be prepared by immunizing a suitable subject (e.g., chicken, rabbit, goat, mouse, or other mammal) with a biomarker protein immunogen. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an ELISA using immobilized biomarker protein. At an appropriate time after immunization, e.g., when the antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique. The technology for producing hybridomas is well known (see generally Coligan et al., eds. (1994) *Current Protocols in Immunology* (John Wiley & Sons, Inc., New York, N.Y).

As described herein above, detection of antibody binding can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S, or ^{3}H.

The antibodies used to practice the invention are selected to have high specificity for the biomarker proteins of interest. Methods for making antibodies and for selecting appropriate antibodies are known in the art.

In other embodiments, the expression of a biomarker of interest is detected at the nucleic acid level. Nucleic acid-based techniques for assessing expression are well known in the art and include, for example, determining the level of biomarker mRNA in a sample. Many expression detection methods use isolated RNA. Any RNA isolation technique that does not select against the isolation of mRNA can be utilized for the purification of RNA from sample cells (see, e.g., Ausubel et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, New York 1987-1999). Additionally, large numbers of tissue samples can readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski (1989, U.S. Pat. No. 4,843,155).

The term "probe" refers to any molecule that is capable of selectively binding to a specifically intended target biomolecule, for example, a nucleotide transcript or a protein encoded by or corresponding to a biomarker. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled.

Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, and antibodies.

Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length cDNA, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to an mRNA or genomic DNA encoding a biomarker of the present invention. Hybridization of an mRNA with the probe indicates that the biomarker in question is being expressed.

In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in detecting the level of mRNA encoded by the biomarkers of the present invention.

An alternative method for determining the level of biomarker mRNA in a sample involves the process of nucleic acid amplification, e.g., by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Pat. No. 4,683,202), ligase chain reaction (Barany, 1991, Proc. Natl. Acad. Sci. USA, 88:189-193), self sustained sequence replication (Guatelli et al., 1990, Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh et al., 1989, Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi et al., 1988, Bio/Technology 6:1197), rolling circle replication (Lizardi et al., U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

Kits for practicing the methods of the invention are further provided. By "kit" is intended any manufacture (e.g., a package or a container) comprising at least one reagent, e.g., an antibody, a nucleic acid probe, etc. for specifically detecting the expression of a biomarker of the invention. The kit may be promoted, distributed, or sold as a unit for performing the methods of the present invention. Additionally, the kits may contain a package insert describing the kit and methods for its use. Any or all of the kit reagents may be provided

within containers that protect them from the external environment, such as in sealed containers or pouches.

In a particular embodiment, the immunochemistry kits of the invention additionally comprise at least two reagents, e.g., antibodies, for specifically detecting the expression of at least two distinct biomarkers. Each antibody may be provided in the kit as an individual reagent or, alternatively, as an antibody cocktail comprising all of the antibodies directed to the different biomarkers of interest.

In an advantageous embodiment, kits for practicing the immunochemistry methods of the invention, particularly the "sandwich" ELISA technique, are provided. Such kits are compatible with both manual and automated immunochemistry techniques. These kits comprise at least one primary capture antibody directed to a biomarker of interest, a labeled secondary detection antibody that is specific for a distinct antigenic site on the biomarker, and chemicals for the detection of the antibody binding to the biomarker. The primary capture antibody may be provided in solution for subsequent attachment to a solid support. Alternatively, the capture antibody may be provided in a kit already bound to a solid support, such as a bead or the well of a microtiter plate. Any chemicals that detect antigen-antibody binding may be used in the practice of the invention. In some embodiments, a secondary detection antibody is conjugated to an enzyme that catalyzes the calorimetric conversion of a substrate. Such enzymes and techniques for using them in the detection of antibody binding are well known in the art. In an advantageous embodiment, the kit comprises a secondary detection antibody that is conjugated to HRP. Substrates, particularly chromogens, compatible with the conjugated enzyme (e.g., tetramethylbenzidine in the case of an HRP-labeled secondary detection antibody) and solutions, such as sulfuric acid, for stopping the enzymatic reaction may be further provided. In particular embodiments, chemicals for the detection of antibody binding comprise commercially available reagents and kits.

Positive and/or negative controls may be included in the kits to validate the activity and correct usage of reagents employed in accordance with the invention. Controls may include samples, such as tissue sections, cells fixed on glass slides, etc., known to be either positive or negative for the presence of the biomarker of interest. In a particular embodiment, the positive control is a solution comprising a biomarker protein of interest. The design and use of controls is standard and well within the routine capabilities of those of ordinary skill in the art.

In other embodiments, kits for identifying biomarker overexpression at the nucleic acid level are further provided. Such kits comprise, for example, at least one nucleic acid probe that specifically binds to a biomarker nucleic acid or fragment thereof.

As discussed above, AKT acts as a key transcriptional regulator of sCLU. It is therefore further contemplated that in aspects of the invention upon detection of activated AKT, or its

overexpression, treatment will commence resulting in the reduction of sCLU expression. Targets of the treatment can include AKT, STAT1 and sCLU, as well as combinations thereof. Inhibition of any one of these molecules is shown herein to reduce sCLU, thereby restoring the efficacy of chemotherapy.

The term "administration" and variants thereof (e.g., "administering" a compound) in reference to a compound of the invention means introducing the compound or a prodrug of the compound into the system of the animal in need of treatment. When a compound of the invention or prodrug thereof is provided in combination with one or more other active agents (e.g., a cytotoxic agent, etc.), "administration" and its variants are each understood to include concurrent and sequential introduction of the compound or prodrug thereof and other agents.

As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts.

The term "therapeutically effective amount" as used herein means that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue, system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician. In reference to cancers or other unwanted cell proliferation, an effective amount comprises an amount sufficient to cause a tumor to shrink and/or to decrease the growth rate of the tumor (such as to suppress tumor growth) or to prevent or delay other unwanted cell proliferation. In some embodiments, an effective amount is an amount sufficient to delay development. In some embodiments, an effective amount is an amount sufficient to prevent or delay occurrence and/or recurrence. An effective amount can be administered in one or more doses. In the case of cancer, the effective amount of the drug or composition may: (i) reduce the number of cancer cells; (ii) reduce tumor size; (iii) inhibit, retard, slow to some extent and preferably stop cancer cell infiltration into peripheral organs; (iv) inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; (v) inhibit tumor growth; (vi) prevent or delay occurrence and/or recurrence of tumor; and/or (vii) relieve to some extent one or more of the symptoms associated with the cancer.

The term "treating cancer" or "treatment of cancer" refers to administration to a mammal afflicted with a cancerous condition and refers to an effect that alleviates the cancerous condition by killing the cancerous cells, but also to an effect that results in the inhibition of growth and/or metastasis of the cancer.

As used herein, "treatment" refers to obtaining beneficial or desired clinical results. Beneficial or desired clinical results include, but are not limited to, any one or more of: alleviation of one or more symptoms (such as tumor growth or metastasis), diminishment of extent of cancer, stabilized (i.e., not worsening) state of cancer, preventing or delaying spread (e.g.,

metastasis) of the cancer, preventing or delaying occurrence or recurrence of cancer, delay or slowing of cancer progression, amelioration of the cancer state, and remission (whether partial or total). The methods of the invention contemplate any one or more of these aspects of treatment.

A "subject in need of treatment" is a mammal with cancer that is life-threatening or that impairs health or shortens the lifespan of the mammal.

A "pharmaceutically acceptable" component is one that is suitable for use with humans and/or animals without undue adverse side effects (such as toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio.

A "safe and effective amount" refers to the quantity of a component that is sufficient to yield a desired therapeutic response without undue adverse side effects (such as toxicity, irritation, or allergic response) commensurate with a reasonable benefit/risk ratio when used in the manner of this invention.

A "pharmaceutically acceptable carrier" is a carrier, such as a solvent, suspending agent or vehicle, for delivering the compound or compounds in question to the animal or human. The carrier may be liquid or solid and is selected with the planned manner of administration in mind. Liposomes are also a pharmaceutical carrier. As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated.

Compositions

API-2 or Triciribine (TCN) (see also triciribine 5'-phosphate (TCN-P), and the DMF adduct of triciribine (TCN-DMF)) is a known compound.

As used in the claims, API-2 refers generally to TCN, TCN-P, TCN-DMF, and pharmaceutically acceptable salts and prodrugs thereof. TCN may be synthesized as described in Tetrahedron Letters, vol. 49, pp. 4757-4760 (1971). TCN-P may be prepared as described in U.S. Pat. No. 4,123,524. TCN-DMF is described in INSERM, vol. 81, pp. 37-82 (1978).

Although the exact dosage of TCN, TCN-P, TCN-DMF, or a pharmaceutically acceptable salt thereof to be administered will vary according to the size and condition of the patient, a suitable dosage range is 15 to 350 mg/m^2 of body surface, preferably 15 to 96 mg/m^2 of body surface, most preferably 25 to 50 mg/m^2 of body surface.

The TCN, TCN-P, TCN-DMF, or pharmaceutically acceptable salt thereof may be administered according to the present invention by any suitable route, such as intravenously, parenterally, subcutaneously, intramuscularly, or orally. The TCN, TCN-P, TCN-DMF, or pharmaceutically acceptable salt thereof may be administered in any conventional form such as a pharmaceutical composition. Suitable pharmaceutical compositions are those containing, in addition to TCN, TCN-P, TCN-DMF, or pharmaceutically acceptable salt thereof, a pharmaceutically acceptable carrier, such as water, starch, sugar, etc. The composition may also contain flavoring agents and may take the form of a solution, tablet, pill, capsule, etc. The ratio of the weight of TCN, TCN-P, TCN-DMF, or pharmaceutically acceptable salt thereof to the weight of the pharmaceutical composition may, of course, vary but is suitably within 1:1 to 1:5000.

For purposes of the present invention, the term pharmaceutically acceptable salt thereof refers to any salt of TCN, TCN-P, or TCN-DMF which is pharmaceutically acceptable and does not greatly reduce or inhibit the activity of TCN, TCN-P, or TCN-DMF. Suitable examples for TCN and TCN-DMF include acid addition salts, with an organic or inorganic acid such as acetate, tartrate, trifluoroacetate, lactate, maleate, fumarate, citrate, methane sulfonate, sulfate, phosphate, nitrate, or chloride. Suitable examples of salts for TCN-P include those in which one or more of the acidic phosphate hydrogens has been replaced with an ion, such as sodium, potassium, calcium, iron, ammonium, or mono-, di- or tri-lower-alkyl ammonium, in addition to the acid addition salts described above. It is to be further understood that the terms TCN, TCN-P, TCN-DMF, and pharmaceutically acceptable salts thereof include all the hydrated forms of these compounds as well as the anhydrous forms.

The taxane are diterpenes produced by the plants of the genus *Taxus* (yews). They were first derived from natural sources, but some have been synthesized artificially. Taxanes include paclitaxel, docetaxel, larotaxel, ortataxel and tesetaxel. Taxanes have been used to produce various chemotherapeutic drugs. The principal mechanism of the taxane class of drugs is the disruption of microtubule assembly and function. It does this by stabilizing GDP-bound tubulin in the microtubule. Microtubules are essential to cell division, and thus by blocking the microtubules the taxane is able to prevent proliferation. Taxanes, including paclitaxel and docetaxal, are known in the art, as is their application and dosaging.

Antisense oligonucleotides inhibitory for clusterin, such as OGX-011, are know in the art and manners of making and using the same are taught in US Patent No. 6,383,808 to Monia, and US Patent Nos. 7,534,773; 7,368,436 and 7,285,541 to Gleave. siRNA knockdown of clusterin is also known in the art [Sowery RD, et al. *BJU Int* (2008) 102:389-397].

Dosage

A person of ordinary skill in the art can easily determine an appropriate dose of one of the

instant compositions to administer to a subject without undue experimentation. Typically, a physician will determine the actual dosage which will be most suitable for an individual patient and it will depend on a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the individual undergoing therapy. The dosages disclosed herein are exemplary of the average case. There can of course be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in cell culture, molecular genetics, nucleic acid chemistry, hybridisation techniques and biochemistry). Standard techniques are used for molecular, genetic and biochemical methods. See, generally, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. and Ausubel et al., Short Protocols in Molecular Biology (1999) 4th Ed, John Wiley & Sons, Inc.; as well as Guthrie et al., Guide to Yeast Genetics and Molecular Biology, Methods in Enzymology, Vol. 194, Academic Press, Inc., (1991), PCR Protocols: A Guide to Methods and Applications (Innis, et al. 1990. Academic Press, San Diego, Calif.), McPherson et al., PCR Volume 1, Oxford University Press, (1991), Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed. (R. I. Freshney. 1987. Liss, Inc. New York, N.Y.), and Gene Transfer and Expression Protocols, pp. 109-128, ed. E. J. Murray, The Humana Press Inc., Clifton, N.J.).

The following examples are offered by way of illustration and not by way of limitation.

Example 1 - Increased activated-AKT and sCLU levels in docetaxel-resistant prostate tumor cells

Differences in AKT levels of parental and docetaxel-resistant paired prostate tumor cell lines were examined [Patterson SG, et al. *Oncogene* (2006) 45:6113-22]. DU145 and PC3, and their matched drug-resistant sublines, were lysed and analyzed for the presence of both total and active phosphorylated (p)AKT. Western blot analysis showed that levels of AKT phosphorylated at amino acids T308 and S473 were significantly higher in the DU145-DR and PC3-DR cell lines when compared to their respective parental lines, while levels of total AKT remained constant (Fig.1). The expression of the sCLU isoform correlated with pAKT levels, being low or undetectable in parental cell lines and rising significantly in the drug-resistant cell lines. Parental PC3 cells already have moderate levels of pAKT because of a PTEN mutation in these cells [Vlietstra RJ, et al. *Cancer Res* (1998) 13:2720-3], and likewise have a slightly higher level of sCLU when compared to the DU145 parental line. In contrast, pERK

expression did not parallel sCLU levels in DR cells and actually decreased, suggesting that AKT but not ERK may be linked to sCLU expression.

Example 2 – Inhibition of AKT function causes a reduction in sCLU expression and enhances chemosensitivity to docetaxel

To investigate if AKT phosphorylation was linked to the upregulation of sCLU seen in the drug-resistant cell lines, DU145-DR and PC3-DR tumor cells were treated with increasing concentrations, from 10 – 40 µmol/L, of a recently identified specific AKT inhibitor, API-2 [Yang L, et al. *Cancer Res* (2004) 13:4394-9], and then evaluated for the presence of sCLU. Western blot analysis confirmed that API-2 treatment was highly efficient in suppressing AKT phosphorylation, as depicted by pAKT-T308 and pAKT-S473 (Fig. 2A). A dose-dependent loss of pAKT was obtained with increasing concentrations of API-1. Most importantly, analysis of the same cells showed that API-2 treatment also down-regulated sCLU expression in a dose dependent manner. In order to ensure that API-2 was specific for AKT, ERK activation was measured and it was found that ERK was not suppressed. Instead, ERK was highly activated by treatment with API-2. These observations on ERK activation are in agreement with the data from others showing that AKT negatively modulates ERK function [Lee JT, et al. *Cell Cycle* (2008) 5:631-6].

We next analyzed if inhibition of AKT activation, and subsequent loss of sCLU expression, would alter cell survival and resistance to docetaxel in the cell lines. The docetaxel-resistant cell lines, DU145-DR and PC3-DR, were pretreated with either (1) medium, (2) DMSO, (3) 20 µmol/L of API-2 or (4) 40 µmol/L of API-2 and then the treated groups were exposed to docetaxel-containing medium. After 48 hours, apoptosis levels were analyzed by AnnexinV-PI staining (Fig. 2B). As indicated by the flow cytometry data, treatment with medium alone did not cause any significant apoptosis, even when exposed to docetaxel, because of the acquired resistance of the cells. However, API-2 plus docetaxel treatment significantly increased the number of apoptotic (AnnexinV+) and necrotic (AnnexinV+ PI+ and PI+) cells in a dose-dependent manner. Thus, API-2 can decrease sCLU (Fig.2A) and at the same time chemosensitize tumor cells to docetaxel (Fig. 2B). From these results, it can be concluded that AKT is necessary to induce sCLU which confers cytoprotection against docetaxel.

Example 3 – Dominant-negative AKT downregulates sCLU expression

Another approach to investigate the contribution of AKT to sCLU expression is by depletion of AKT function via dominant-negative (DN) constructs. To confirm that AKT controls clusterin expression, we transfected HA-tagged DN-AKT into our docetaxel resistant cell lines [Jiang K, et al. *Mol Cell Biol* (2000) 1:139-48] and reanalyzed them for sCLU expression (Fig. 3). The efficiency of transfection was monitored by GSK-3β phosphorylation, which is the direct downstream target of AKT. Fig. 3A shows that only DN-AKT, but not control PCDNA-3

transfected DU145-DR or PC3-DR tumor cells, had markedly reduced p-GSK-3β, while the total GSK-3β levels were constant and unaffected by these treatments. The presence of the transfected DN-AKT was monitored by western blotting with anti-HA because DN-AKT is tagged with this marker. Western blotting with anti-AKT shows a darker band in DN-AKT-transfected cells because of the addition of the overexpressed construct to the constitutive levels of AKT. These analyses demonstrated that HA-tagged DN-AKT was efficiently expressed and it markedly suppressed GSK-3β phosphorylation, confirming its function integrity. We then probed for the effect of DN-AKT expression on sCLU induction. Overexpression of DN-AKT, but not control PCDNA3 vector, in both DU145-DR and PC3-DR cells effectively suppressed CLU protein expression (Fig.3A). As a control ERK was evaluated and knockdown of AKT was again seen to enhance ERK activation, which mirrors the results from API-2 treated cells in Fig. 2A.

The above data indicated that sCLU protein was downregulated by the loss of AKT function. In order to identify if the downregulation of clusterin protein levels brought upon by inhibiting AKT function is mediated at the level of transcription, assays were performed for full-length CLU mRNA levels in DMSO-treated and API-2 treated, or PCDNA3 control and DN-AKT transfected cells (Fig. 3B). RT-PCR analysis indicated that inhibition of AKT function by DN-AKT expression dramatically decreased sCLU gene expression levels as compared to control PCDNA3-transfection. Confirmation was also provided by the observation that API-2 treated tumor cells expressed no sCLU mRNA in comparison to DMSO-treated control tumor cells. As a control for equal loading, GADPH mRNA was also measured and showed a consistent level of expression in all groups.

Example 4 – DN-AKT chemosensitizes tumor cells to docetaxel via caspase-3-dependent apoptosis

In order to identify if the docetaxel sensitivity following API-2 treatment or transfection of DN-AKT was mediated via caspase 3 dependent apoptosis, the cells were analyzed using the Active Caspase-3 FITC MAb Apoptosis Kit. All groups before and after API-2 treatment or DN-AKT transfection, accompanied by DMSO and PCDNA3 controls, were placed in docetaxel medium for 24 h and subsequently evaluated for caspase 3 activation. Fig. 4A indicated that DMSO-treated and control PCDNA3 vector-transfected DU145-DR and PC3-DR tumor cells did not express active caspase 3 when exposed to docetaxel, which is consistent with their acquired property of drug resistance. On the other hand, levels of active caspase 3 were extremely elevated following API-2 treatment or DN-AKT transfection in both DU145-DR and PC3-DR cell lines. These elevated levels returned to near basal levels when the cells were pretreated with the broad range caspase inhibitor, zVAD.fmk (Fig. 4A). However, the caspase-1 specific inhibitor, zVAD.amc, could not diminish active caspase-3.

Thus, caspase 3-dependent apoptosis is responsible for the detrimental effect resulting from loss of AKT function induced by API-2. Visual analysis of docetaxel sensitivity of these cells was monitored by methylene blue staining and it confirmed that treatment with API-2 or transfection with DN-AKT restored docetaxel sensitivity and showed few cells surviving, while DMSO control and vector-transfected cells remained resistant and showed unimpeded growth (Figure 4B). Taken together, this data lends concrete evidence to the importance of activated AKT in mediating docetaxel resistance.

Example 5 – sCLU expression alone is sufficient to develop the docetaxel-resistant phenotype

It is possible that AKT can induce other prosurvival proteins unrelated to sCLU to mediate chemoresistance in tumor cells. In order to identify if sCLU expression alone can produce docetaxel resistance in our cell lines, we transfected a full length Myc-tagged sCLU construct into DU145-DR cells before treatment with API-2. Then the cells were exposed to docetaxel. Western blot analysis confirms sCLU overexpression in both control and API-2 transfected cells transfected with the myc-tagged protein (Fig. 5A). Upon API-2 treatment, sCLU is lost in PCDNA3 control vector-transfected cells but not in sCLU-overexpressing cells. The effectiveness of API-2 was monitored by the presence of pAKT at S473 and T308 and it was confirmed that API-2 markedly reduced pAKT levels. These cells were then examined for sensitivity to docetaxel to determine if sCLU is overexpressed, the tumor cells should survive in docetaxel, even under API-2 treatment (Fig. 5B). The results show that API-2 treatment can chemosensitize PCDNA3-control vector transfected cells but cannot do so in sCLU-overexpressing tumor cells. Thus, we can conclude that sCLU can overcome API-2 induced cell death and is a key mediator of AKT-mediated docetaxel resistance.

Example 6 – Inhibition of AKT results in suppression of STAT1 activation

STAT1 has been shown to be critical for the upregulation of sCLU in docetaxel-resistant tumor cells [Patterson SG, et al. *Oncogene* (2006) 45:6113-22]. This, combined with the showing above that AKT is also required for sCLU induction, led to an investigation in the linkage, if any, between AKT and STAT1. Therefore we analyzed for active STAT1 in DU145DR tumor cells before and after suppression of AKT function (Fig. 6). DU145DR was found to express markedly higher phospho-STAT1 levels than in the parental DU145 tumor cells. Most importantly, suppression of AKT function either via DN-AKT transfection or API-2 treatment abolished the presence of phospho-STAT1, indicating that AKT does control STAT1 function. Thus, AKT appears to be upstream of STAT1, which has been shown to be a transcription factor necessary for sCLU gene transcription [Patterson SG, et al. *Oncogene* (2006) 45:6113-22].

Example 7 – An AKT-clusterin Pathway Correlates with Advanced Disease in Prostate Cancer

To validate the AKT-clusterin pathway clinically, fourty-five (45) resection specimens were procured from prostate cancer patients, with 12 samples each from Gleason 6 (well-differentiated), Gleason 7 (intermediate) and Gleason 8+ (poorly-differentiated) grades, including 9 metastatic tumors. Clinical stages of disease in these patients include primary tumors localized to the prostate (Stage I) and progressing to lymph node and distant metastasis (Stage IV). Immunohistochemistry showed a high correlation of pAKT with cytoplasmic clusterin, representing the isoform reported to be anti-apoptotic. As seen in FIG. 7A, the low grade Gleason 6 tumor had minimal expression of pAKT and clusterin, while the Gleason 8+ and metastatic tumors had high expression of both. Comparison of the various grades of tumors revealed that there is a steady rise in pAKT and clusterin expression with advancing disease and the presence of pAKT tends to be accompanied by the presence of clusterin (FIG. 7B). Using a scoring system of 1-9, which represents the product of the staining intensity (0-3) with the percentage of positive tumor cells (0-3), the Gleason 6 tumors had a mean (S.D.) of 0.75 (1.14) for pAKT and 0.67 (0.89) for clusterin (FIG 7C). Gleason 7 tumors showed 1.5 (1.31) for pAKT and 1.5 (0.90) for clusterin. Gleason 8+ tumors showed 4.25 (1.22) for pAKT and 3.33 (1.37) for clusterin. Lastly, metastatic tumors showed 6.22 (1.79) for pAKT and 5.33 (1.0) for clusterin. Statistical analysis indicated a Spearman's correlation coefficient, $r = 0.73$ (p-value<0.0001) between clusterin and pAKT, $r = 0.84$ (p-value<0.0001) between clusterin and Gleason score, and $r = 0.84$ (p-value<0.0001) between pAKT and Gleason score. Thus, there is a tight correlation between pAKT and clusterin, while both are correlated with Gleason score.

These two molecules, pAKT and clusterin, were next compared with stage of tumors. Among the 45 patients, 28 Stage I, 2 Stage II, 4 Stage III, and 11 Stage IV tumors could be recorded. The Spearman's correlation coefficient for Stage versus clusterin, $r = 0.86$, $p<0.0001$ and for Stage versus pAKT, $r = 0.76$, $p<0.0001$, denotes a strong, positive correlation between clusterin and Stage, as well as between pAKT and Stage.

These results demonstrate that pAKT and clusterin are correspondingly increased with advancing grade or stage of disease. Accordingly, hese two markers can be utilized to predict docetaxel responsiveness. Docetaxel effectiveness exhibits propensity to be lost with tumor progression. The acquisition of high pAKT levels, leading to high production of cytoplasmic clusterin, may induce drug resistance and become a barrier to successful therapy for these patients. Secondly, positive screening of these molecular markers could divert treatment towards drugs that selectively target AKT and/or clusterin. AKT inhibitors such as perifosine and siRNA-clusterin are already in early phase clinical trials and they could improve cancer

therapy in combination with docetaxel. This strategy would also benefit other cancers, including breast, colon, lung, kidney, and bladder, which are often treated with docetaxel and are also reported to display clusterin overexpression in late-stage tumors [Shannan B, et al. *Cell Death Differ* (2006) 1:12-9].

MATERIALS AND METHODS

Antibodies and Reagents:

Mouse monoclonal antibodies to Phospho-AKT(Ser473), Phospho-AKT(Thr308), AKT, Phospho-p44/42 ERK, Phospho-GSK-3β and GSK, Phospho- STAT1 and STAT1 were from Cell Signaling Technology (Beverly, MA). Mouse monoclonal anti-human CLU was from Upstate Biotechnology (Lake Placid, NY). Monoclonal anti-ERK was obtained from Transduction Laboratories (San Diego, CA) and monoclonal anti-β-actin was from Sigma (St. Louis, MO). The AKT-specific inhibitor, API-2, was provided by Dr. Jin Cheng, H. Lee Moffitt Cancer Center, Tampa, FL [Yang L, et al. *Cancer Res* (2004) 13:4394-9].

Cell Culture and Selection of Docetaxel-Resistant (DR) Clones:

Androgen-independent DU145 and PC3 prostate tumor cell lines (American Type Culture Collection (Rockville, MD) were maintained in RPMI 1640 medium containing 10% heat-inactivated FBS with 100 units/mL penicillin, 100-μg/mL streptomycin. Docetaxel-resistant (DR) cell lines of DU145 and PC3 were developed as previously described [Patterson SG, et al. *Oncogene* (2006) 45:6113-22]. Briefly, the cells surviving initial culture in 1 nmol/L docetaxel were passaged 4 times prior to increase of docetaxel to 5.5 nmol/L and subsequently to 11 nmol/L in the culture medium. Cells were then maintained continuously in 11 nmol/L docetaxel and labeled DU145-DR and PC3-DR.

Western Blotting Analysis:

Tumor cells, seeded at 5 x 10^5 cells/well in a six-well plate, were untreated or treated with DMSO, 10 μmol/L, 20 μmol/L or 40 μmol/L of API-2 for 2 h at 37°C. They were subsequently cultured for 48 h at 37°C in 11 nmol/L docetaxel-containing medium. Cells were then solubilized by incubation at 4°C for 30 min in 1% NP-40, 10 mmol/L Tris, 140 mmol/L NaCl, 0.1 mmol/L PMSF, 10 mmol/L iodoacetamide, 50 mmol/L NaF, 1 mmol/L EDTA, 1 mmol/L sodium orthovanadate, 0.25% Na Deoxycholate, 100 μl ALA, and 100 μl of phosphatase inhibitor cocktails I and II (Sigma, St. Louis, MO). Whole cell lysates were centrifuged at 12,000 x g for 10 min to remove nuclei and cell debris. The protein concentration of the soluble extracts was determined by using the Bio-Rad (Bradford) protein assay (Bio-Rad, Hercules, CA). Separation of 50 μg of total protein was performed on 10% SDS-polyacrylamide gels, and transferred to a nitrocellulose membrane before immunoblotting with primary antibodies specific for phospho-AKT, phopspho-ERK or CLU. Equal loading controls

were performed by blotting with antibodies to unphosphorylated forms of the same proteins or with β-actin.

Dominant-negative (DN) AKT or sCLU Plasmid Transfection:

Transfection of DN-AKT [Jiang K, et al. *Mol Cell Biol* (2000) 1:139-48] into docetaxel-resistant PC3 and DU145 cells were performed using the standard lipofectAMINE protocol (Invitrogen-Life Technologies, Inc). Briefly, 5 x 10^5cells were plated into each well of a 6 well plate 24 h before transfection with 4 μg of hemaglutinin (HA)-tagged DN-AKT plasmid or PCDNA3 control plasmid. After culture for an additional 48 h in 11nmol/L docetaxel-containing medium, the transfected cells were washed twice with PBS. Cell lysates were prepared and loaded on to lanes of a 10% SDS-polyacrylamide gel. Effectiveness of DN-AKT transfection was evaluated by immunoblotting with anti-HA as well as anti-AKT. Then, the lysates were immunoblotted with primary antibodies against phospho-GSK-3β to ensure functional deletion of AKT, which normally directly phosphorylates GSK-3β. Aliquots of the same cell lysates were also run in parallel and immunoblotted for CLU or phospho-ERK. Total GSK-3β and ERK were also evaluated.

Transfection of myc-tagged sCLU [Trougakos IP, et al. *Free Radic Biol Med* (2005) 4:436-49] or its control vector, PCDNA3, into DU145-DR tumor cells were performed using the same lipfectAMINE protocol. Transfected cells were lysed and probed with anti-myc as well as anti-CLU to visualize efficient transfection. The lysates were then analyzed by western blotting for the presence of phospho-AKT and total AKT. Equal loading was monitored by β-actin.

Apoptosis Assays:

PC3-DR and DU145-DR tumor cells, seeded overnight in a six well plate at 5 x 10^5 cells/well were exposed to 10 - 40 μmol/L of API-2 (or DMSO as control) for 2 h at 37°C and further cultured in 11 nmol/L docetaxel-containing medium for 24 h at 37°C. The cells were then analyzed by flow cytometry using the Annexin V-PI Apoptosis Kit (BD Pharmagen, Franklin Lakes, NJ). Each well was trypsinized and resuspended in 1x binding buffer at a concentration of 5x 10^5 cells/ml and stained with Annexin-FITC and Propidium Iodide (PI).

To determine caspase 3 activation, 5 x 10^5 tumor cells seeded in each well of a six-well plate were treated with DMSO or 40 μM API-2, with or without 50 μM of the broad range caspase inhibitor, zVAD.fmk (EMD Biosciences, San Diego, CA) or a caspase-1 specific inhibitor, zVAD.AMC (Bachem, Torrance, CA). In addition, instead of API-2 treatment to suppress AKT function, DN-AKT-transfected tumor cells were also used and PCDNA3-transfected tumor cells served as the vector control. These transfected cells were treated with or without zVAD.fmk or zVAD.AMC. The various groups of cells were then cultured 24 h at 37°C in 11

nmol/L docetaxel, after which the cells were trypsinized and assayed by flow cytometry for active-caspase-3 (Active Caspase-3 FITC MAb Apoptosis Kit, BD Pharmagen).

Methylene Blue Staining

After AKT inhibition, either via API-2 treatment or DN-AKT expression, the cells were further cultured in docetaxel-containing medium for 72 h at 37°C. To assess cell growth and survival, the medium was discarded before the cells were fixed with methanol for 3 min at room temperature, followed by staining with methylene blue for 2 min. The stained cells were washed twice with deionized water and allowed to dry overnight. Images were observed with a Leitz Orthoplan 2 microscope (Photometrics Ltd, Tucson, AZ), and pictures were captured by a CCD camera with the Smart Capture Program (Vysis, Downers Grove, IL).

Isolation of RNA and RT-PCR:

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was used to determine mRNA expression for sCLU in DU145-DR and PC3-DR cells. Briefly, total RNA was prepared using TRIzol reagent (Invitrogen, Carlsbad, CA). A total amount of 1 µg of RNA was converted to cDNA by Omniscript reverse transcriptase in a solution containing random hexanucleotide, dNTP, Rnase inhibitor and RT-buffer (Qiagen, Valencia, CA). Aliquots of 1 µl of DNA resulting from each RT reaction were then subjected to PCR. The temperature profiles of PCR were as follows: an initial denaturation step of 94°C for 5 min, followed by 25 cycles of 94°C for 15 s, 50°C for 15 s, 72°C for 30s, and a final elongation step of 72°C for 7 min. PCR was performed in reactions containing Taq DNA polymerase, dNTP PCR buffer, and the sCLU primer (Sense 5'-CTTGATGCCCTTCTCTCCGTA-3' [SEQ ID NO: 1]; Antisense 5'-AACGTCCGAGTCAGAAGTGTG-3' [SEQ ID NO: 2]). As a control, expression of human GAPDH mRNA expression was evaluated, using sense primer (5'-CAAAAGGGTCATCATCTCTGC-3' [SEQ ID NO: 3]) and antisense primer (5'-GAGGGGCCATCCACAGTCTTC-3' [SEQ ID NO: 4]). RT-PCR products were analyzed by agarose gel electrophoresis.

Immunohistochemistry:

Paraffin-embedded prostate tumor samples from early to advanced grades of disease (Gleason 6-8+), including metastasis, were obtained from the Tissue Core under an approved IRB protocol without any name identification. These samples were immunostained for expression of clusterin and pAKT, along with isotype controls as negative controls in the place of primary antibody in each experiment. The immunohistochemical staining was performed manually at room temperature, using the avidin-biotin-peroxidase complex method (Vectastatin Elite ABC kit; Vector lab). Briefly, pretreatment for antigen retrieval with a pressure cooker involved heating the slides with a microwave oven in 250 ml of unmasking

solution (Vector Lab) for 10 min at a high power level, followed by 20 min of cooling. Endogenous peroxidase and nonspecific background staining were blocked by incubating slides with 50:50 solution of 3% hydrogen peroxide and methanol for 20 min. After washing with PBS for 5 min, slides were blocked with fetal calf serum for 20 min, followed by incubation with the primary monoclonal antibodies for Stat1, Stat3 (Cell Signaling, Beverly, MA) and clusterin (Upstate Cell Signaling Solutions (Waltham, MA) at a dilution of 1:100, for 2 h at room temperature. After rinsing with PBS for 5 min, slides were incubated with a biotinylated secondary antibody for 30 min and washed again. After washing with PBS for 5 min, slides were incubated with avidin-biotin complex for 30 min. and washed again. The slides were developed with 3,3-diaminobenzidine (DAB Substrate kit for peroxidase) (Vector lab). All of the slides were lightly counterstained with hematoxylin for 10 s before dehydration and mounting. Immunostaining was observed with a Leitz Orthoplan 2 microscope and images were captured by a CCD camera with the Smart Capture Program (Vysis, Downers Grove, IL).

The slides were read blindly by the pathologist (D. Coppola) and decoded after the results were tabulated. The positive reaction of pAKT was scored into four grades according to the intensity of the staining: 0, 1+, 2+, and 3+. The percentage of pAKT-positive cells on each slide was also scored into four categories: 0 (0%), 1 (1% to 33%), 2 (34% to 66%), and 3 (67% to 100%). For each tumor specimen, the product of the intensity score and the percentage of positive cells produced a final Intensity Score ranging from1 to 9.

Statistical Analysis:

Spearman's correlation coefficient was used to assess the correlation between pAKT, clusterin and Gleason score in the patient tumor samples. Gleason score was treated as an ordinal variable with values of 6, 7, 8+ (scored as an 8 for ranking and analysis purposes), and metastasis (scored as a 9 for ranking and analysis purposes). The Holm step-down method was used to adjust for multiple testing and all analyses were performed with SAS software (Cary, NC). The same methodology was used to analyze correlation between pAKT, clusterin and Stage of disease.

All references cited in the present application are incorporated in their entirety herein by reference to the extent not inconsistent herewith.

It will be seen that the advantages set forth above, and those made apparent from the foregoing description, are efficiently attained and since certain changes may be made in the above construction without departing from the scope of the invention, it is intended that all matters contained in the foregoing description or shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

It is also to be understood that the following claims are intended to cover all of the generic and specific features of the invention herein described, and all statements of the scope of the invention which, as a matter of language, might be said to fall therebetween. Now that the invention has been described,

What is claimed is:

1. A method of treating prostate cancer in a patient comprising the steps of:

obtaining a sample of the prostate cancer cell population from the patient;

contacting the sample with at least one antibody, wherein the antibody specifically binds to an activated AKT;

detecting binding of the antibody to the activated AKT protein, wherein binding is indicative of resistance to treatment of the cancer cell with one or more taxane drugs;

pretreating the patient with one or more inhibitors of AKT or sCLU responsive to the detection of binding of the antibody to the activated AKT protein; and

treating the patient with a taxane drug or mitoxantrone.

2. The method according to claim 1 further comprising the step of screening the sample for overexpression of sCLU, wherein overexpression is indicative of resistance to treatment of the cancer cell with taxane drug or mitoxantrone.

3. The method according to claim 2 wherein the overexpression is detected by binding of antibody specific to sCLU.

4. The method according to claim 2 wherein the step of screening for overexpression detects overexpressed sCLU using nucleic acids complementary to the CLU nucleic acid.

5. The method according to claim 4 wherein the nucleic acids are complementary to the sCLU nucleic acid.

6. The method according to claim 1 wherein the one or more inhibitors of sCLU is selected from the group consisting of antisense oligonucleotides and siRNA.

7. The method according to claim 1 wherein the taxane drug is a drug selected from the group consisting of docetaxel and paclitaxel.

8. The method according to claim 1 wherein the AKT inhibitor is selected from the group consisting of an API-2 and perifosine.

9. The method according to claim 1 wherein the one or more inhibitors of sCLU is an oligonucleotide that targets clusterin and that has a sequence complementary to clusterin-encoding mRNA.

10. A method of chemosensitizing a prostate cancer cell population comprising the steps of:

obtaining a sample of the prostate cancer cell population;

contacting the sample with at least one antibody, wherein the antibody specifically binds to an activated AKT;

detecting binding of the antibody to the activated AKT protein, wherein binding is indicative of resistance to treatment of the cancer cell with one or more chemotheraputics; and

contacting the prostate cancer cell population with one or more inhibitors of sCLU or STAT1 responsive to the detection of binding of the antibody to the activated AKT protein.

11. The method according to claim 10 wherein the one or more inhibitors of sCLU is selected from the group consisting of antisense oligonucleotides and siRNA.

12. The method according to claim 10 further comprising the step of contacting the cancer cell population with a taxane drug.

13. The method according to claim 12 wherein the taxane drug is a drug selected from the group consisting of docetaxel and paclitaxel.

14. The method according to claim 10 further comprising the step of contacting the prostate cancer cell population with a chemotherapeutic selected from the group consisting of doxorubicin, camphothecin, cisplatin, 5-fluorouracil, dacarbazine, etoposide, mitoxantrone and TRAIL.

15. The method according to claim 10 wherein the one or more inhibitors of sCLU is an oligonucleotide that targets clusterin and that has a sequence complementary to clusterin-encoding mRNA.

16. The method according to claim 10 further comprising the step of screening the sample for overexpression of sCLU, wherein overexpression is indicative of resistance to treatment of the cancer cell with one or more chemotheraputics.

17. The method according to claim 16 wherein the overexpression is detected by binding of antibody specific to sCLU.

18. The method according to claim 16 wherein the step of screening for overexpression detects overexpressed sCLU using nucleic acids complementary to the CLU nucleic acid.

19. The method according to claim 18 wherein the nucleic acids are complementary to the sCLU nucleic acid.

20. A method of chemosensitizing a prostate cancer cell population comprising the steps of:

obtaining a sample of the prostate cancer cell population;

screening the cancer cell population for overexpression of AKT, wherein overexpression is indicative of resistance to treatment of the cancer cell with one or more chemotheraputics; and

contacting the cancer cell population with one or more inhibitors of sCLU or STAT1 responsive to the detection of overexpression of AKT.

21. The method according to claim 20 wherein the step of screening for overexpression detects overexpressed AKT using nucleic acids complementary to the AKT nucleic acid.

22. The method according to claim 20 wherein the step of screening for overexpression detects overexpressed AKT using antibodies specific for AKT.

23. The method according to claim 20 wherein the one or more inhibitors of sCLU is selected from the group consisting of antisense oligonucleotides and siRNA.

24. The method according to claim 20 further comprising the step of contacting the cancer cell population with a taxane drug or mitoxantrone.

25. The method according to claim 24 wherein the taxane drug is a drug selected from the group consisting of docetaxel and paclitaxel.

26. The method according to claim 20 further comprising the step of contacting the cancer cell population with a chemotherapeutic selected from the group consisting of doxorubicin, camphothecin, cisplatin, 5-fluorouracil, dacarbazine, etoposide, mitoxantrone and TRAIL.

27. The method according to claim 20 wherein the one or more inhibitors of sCLU is an oligonucleotide that targets clusterin and that has a sequence complementary to clusterin-encoding mRNA.

28. The method according to claim 20 further comprising the step of screening the sample for overexpression of sCLU, wherein overexpression is indicative of resistance to treatment of the cancer cell with one or more chemotheraputics.

29. The method according to claim 28 wherein the overexpression is detected by binding of antibody specific to sCLU.

30. The method according to claim 28 wherein the step of screening for overexpression detects overexpressed sCLU using nucleic acids complementary to the CLU nucleic acid.

31. The method according to claim 30 wherein the nucleic acids are complementary to the sCLU nucleic acid.

32. A method for evaluating susceptibility to treatment of prostate cancer with a taxane drug or mitoxantrone in a patient, the method comprising:

obtaining a sample from the patient;

contacting the sample with at least one antibody, wherein the antibody specifically binds to an activated AKT; and

detecting binding of the antibody to the activated AKT protein, wherein binding is indicative of resistance to treatment of the prostate cancer.

33. The method according to claim 32 further comprising the step of administering two or more treatments to the patient responsive to the detection of binding of the antibody to the activated AKT protein.

34. The method according to claim 33 wherein one of the two or more treatments is a drug that selectively targets AKT or clusterin.

35. The method according to claim 34 wherein the AKT inhibitor is selected from the group consisting of an API-2 and perifosine.

36. The method according to claim 34 wherein the clusterin inhibitor is selected from the group consisting of antisense oligonucleotides and siRNA.

37. The method according to claim 33 wherein a second of the two or more treatments is a taxane drug or mitoxantrone.

38. The method according to claim 37 wherein the taxane drug is a drug selected from the group consisting of docetaxel and paclitaxel.

39. The method according to claim 32 further comprising the step of screening the sample for overexpression of sCLU, wherein overexpression is indicative of resistance to treatment of the cancer cell with one or more chemotheraputics.

40. The method according to claim 39 wherein the overexpression is detected by binding of antibody specific to sCLU.

41. The method according to claim 39 wherein the step of screening for overexpression detects overexpressed sCLU using nucleic acids complementary to the CLU nucleic acid.

42. The method according to claim 41 wherein the nucleic acids are complementary to the sCLU nucleic acid.

43. A method for evaluating susceptibility to treatment of prostate cancer with a taxane drug or mitoxantrone in a patient, the method comprising:

obtaining a sample from the patient;

screening the cancer cell population for overexpression of AKT, wherein overexpression is indicative of resistance to treatment of the cancer cell with a taxane drug or mitoxantrone.

44. A method of inducing apoptosis in a docetaxel resistant prostate cancer cell comprising the steps of:

pretreating the cell with an AKT inhibitor; and

treating the cell with docetaxel.

45. The method according to claim 44 wherein the AKT inhibitor is selected from the group consisting of an API-2 and perifosine.

46. A method of treating prostate cancer in a patient comprising the steps of:

obtaining a sample of the prostate cancer cell population from the patient;

contacting the sample with at least one antibody, wherein the antibody specifically binds to sCLU;

detecting binding of the antibody to the sCLU protein, wherein binding is indicative of resistance to treatment of the cancer cell with one or more taxane drugs;

pretreating the patient with one or more inhibitors of AKT or sCLU responsive to the detection of binding of the antibody to the sCLU protein; and

treating the patient with a taxane drug or mitoxantrone.

47. The method according to claim 46 further comprising the step of screening the sample for overexpression of AKT, wherein overexpression is indicative of resistance to treatment of the cancer cell with taxane drug or mitoxantrone.

48. The method according to claim 47 wherein the overexpression is detected by binding of antibody specific to pAKT.

49. The method according to claim 48 wherein the step of screening for overexpression detects overexpressed AKT using nucleic acids complementary to the AKT nucleic acid.

50. The method according to claim 46 wherein the one or more inhibitors of sCLU is selected from the group consisting of antisense oligonucleotides and siRNA.

51. The method according to claim 46 wherein the taxane drug is a drug selected from the group consisting of docetaxel and paclitaxel.

52. The method according to claim 46 wherein the AKT inhibitor is selected from the group consisting of an API-2 and perifosine.

53. The method according to claim 46 wherein the one or more inhibitors of sCLU is an oligonucleotide that targets clusterin and that has a sequence complementary to clusterin-encoding mRNA.

54. A method of chemosensitizing a prostate cancer cell population comprising the steps of:

obtaining a sample of the prostate cancer cell population;

contacting the sample with at least one antibody, wherein the antibody specifically binds to sCLU;

detecting binding of the antibody to the sCLU protein, wherein binding is indicative of resistance to treatment of the cancer cell with one or more chemotheraputics; and

contacting the prostate cancer cell population with one or more inhibitors of sCLU or STAT1 responsive to the detection of binding of the antibody to the sCLU protein.

55. The method according to claim 54 wherein the one or more inhibitors of sCLU is selected from the group consisting of antisense oligonucleotides and siRNA.

56. The method according to claim 54 further comprising the step of contacting the cancer cell population with a taxane drug.

57. The method according to claim 56 wherein the taxane drug is a drug selected from the group consisting of docetaxel and paclitaxel.

58. The method according to claim 54 further comprising the step of contacting the prostate cancer cell population with a chemotherapeutic selected from the group consisting of doxorubicin, camphothecin, cisplatin, 5-fluorouracil, dacarbazine, etoposide, mitoxantrone and TRAIL.

59. The method according to claim 54 wherein the one or more inhibitors of sCLU is an oligonucleotide that targets clusterin and that has a sequence complementary to clusterin-encoding mRNA.

60. The method according to claim 54 further comprising the step of screening the sample for overexpression of AKT, wherein overexpression is indicative of resistance to treatment of the cancer cell with one or more chemotheraputics.

61. The method according to claim 60 wherein the overexpression is detected by binding of antibody specific to AKT.

62. The method according to claim 60 wherein the overexpression is detected by binding of antibody specific to pAKT.

63. The method according to claim 60 wherein the step of screening for overexpression detects overexpressed AKT using nucleic acids complementary to the AKT nucleic acid.

64. A method of chemosensitizing a prostate cancer cell population comprising the steps of:

obtaining a sample of the prostate cancer cell population;

screening the cancer cell population for overexpression of sCLU, wherein overexpression is indicative of resistance to treatment of the cancer cell with one or more chemotheraputics; and

contacting the cancer cell population with one or more inhibitors of sCLU or STAT1 responsive to the detection of overexpression of sCLU.

65. The method according to claim 64 wherein the step of screening for overexpression detects overexpressed sCLU using nucleic acids complementary to the sCLU nucleic acid.

66. The method according to claim 64 wherein the step of screening for overexpression detects overexpressed sCLU using antibodies specific for sCLU.

67. The method according to claim 64 wherein the one or more inhibitors of sCLU is selected from the group consisting of antisense oligonucleotides and siRNA.

68. The method according to claim 64 further comprising the step of contacting the cancer cell population with a taxane drug or mitoxantrone.

69. The method according to claim 68 wherein the taxane drug is a drug selected from the group consisting of docetaxel and paclitaxel.

70. The method according to claim 64 further comprising the step of contacting the cancer cell population with a chemotherapeutic selected from the group consisting of doxorubicin, camphothecin, cisplatin, 5-fluorouracil, dacarbazine, etoposide, mitoxantrone and TRAIL.

71. The method according to claim 64 wherein the one or more inhibitors of sCLU is an oligonucleotide that targets clusterin and that has a sequence complementary to clusterin-encoding mRNA.

72. The method according to claim 64 further comprising the step of screening the sample for overexpression of AKT, wherein overexpression is indicative of resistance to treatment of the cancer cell with one or more chemotheraputics.

73. The method according to claim 72 wherein the overexpression is detected by binding of antibody specific to AKT.

74. The method according to claim 72 wherein the overexpression is detected by binding of antibody specific to pAKT.

75. The method according to claim 72 wherein the step of screening for overexpression detects overexpressed AKT using nucleic acids complementary to the AKT nucleic acid.

76. A method for evaluating susceptibility to treatment of prostate cancer with a taxane drug or mitoxantrone in a patient, the method comprising:

obtaining a sample from the patient;

contacting the sample with at least one antibody, wherein the antibody specifically binds to sCLU; and

detecting binding of the antibody to the sCLU protein, wherein binding is indicative of resistance to treatment of the prostate cancer.

77. The method according to claim 76 further comprising the step of administering two or more treatments to the patient responsive to the detection of binding of the antibody to the sCLU protein.

78. The method according to claim 77 wherein one of the two or more treatments is a drug that selectively targets AKT or clusterin.

79. The method according to claim 78 wherein the AKT inhibitor is selected from the group consisting of an API-2 and perifosine.

80. The method according to claim 78 wherein the clusterin inhibitor is selected from the group consisting of antisense oligonucleotides and siRNA.

81. The method according to claim 77 wherein a second of the two or more treatments is a taxane drug or mitoxantrone.

82. The method according to claim 81 wherein the taxane drug is a drug selected from the group consisting of docetaxel and paclitaxel.

83. The method according to claim 76 further comprising the step of screening the sample for overexpression of AKT, wherein overexpression is indicative of resistance to treatment of the cancer cell with one or more chemotheraputics.

84. The method according to claim 83 wherein the overexpression is detected by binding of antibody specific to AKT.

85. The method according to claim 83 wherein the overexpression is detected by binding of antibody specific to pAKT.

86. The method according to claim 83 wherein the step of screening for overexpression detects overexpressed AKT using nucleic acids complementary to the AKT nucleic acid.

Figure 1

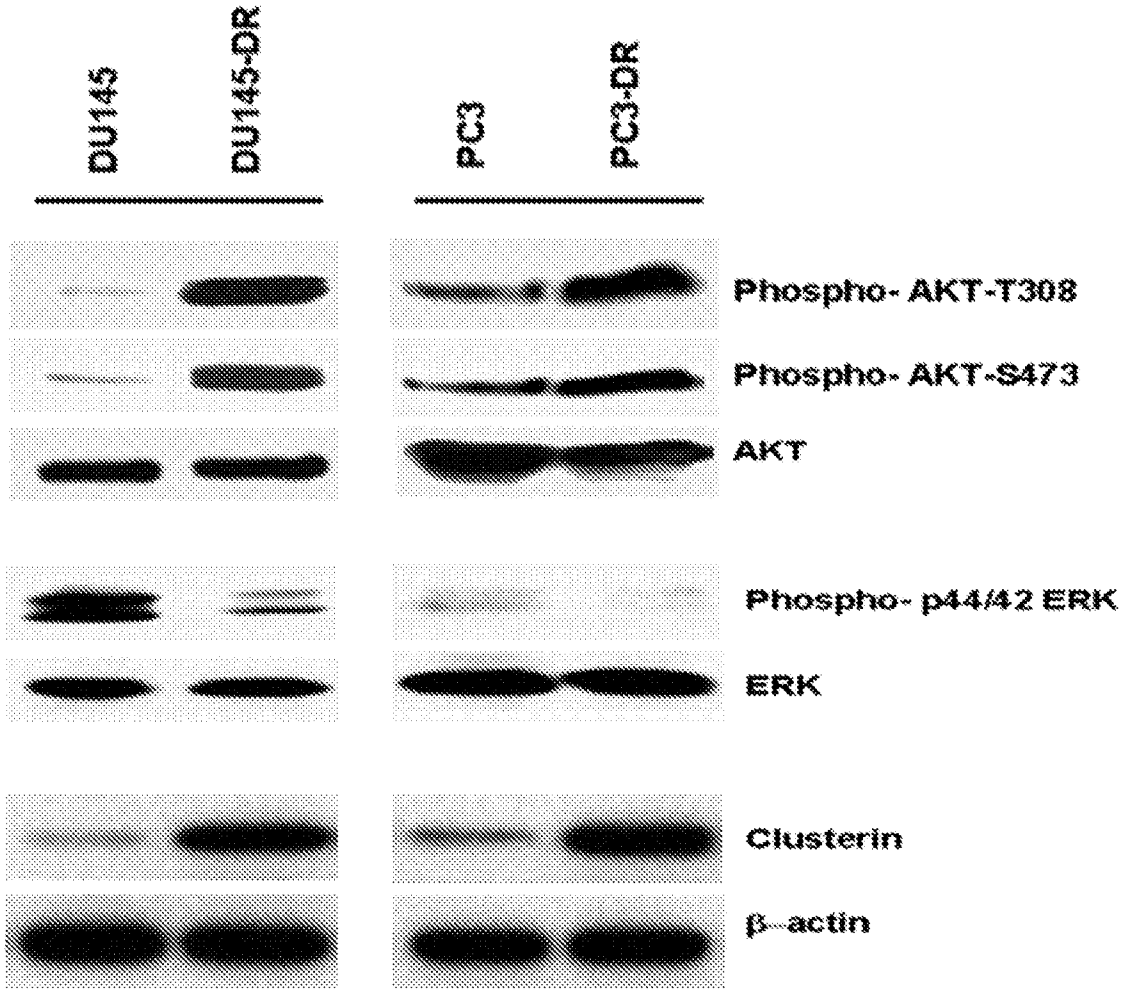

DU145
DU145-DR
PC3
PC3-DR
Phospho- AKT-T308
Phospho- AKT-S473
AKT
Phospho- p44/42 ERK
ERK
Clusterin
β-actin

Figure 2A

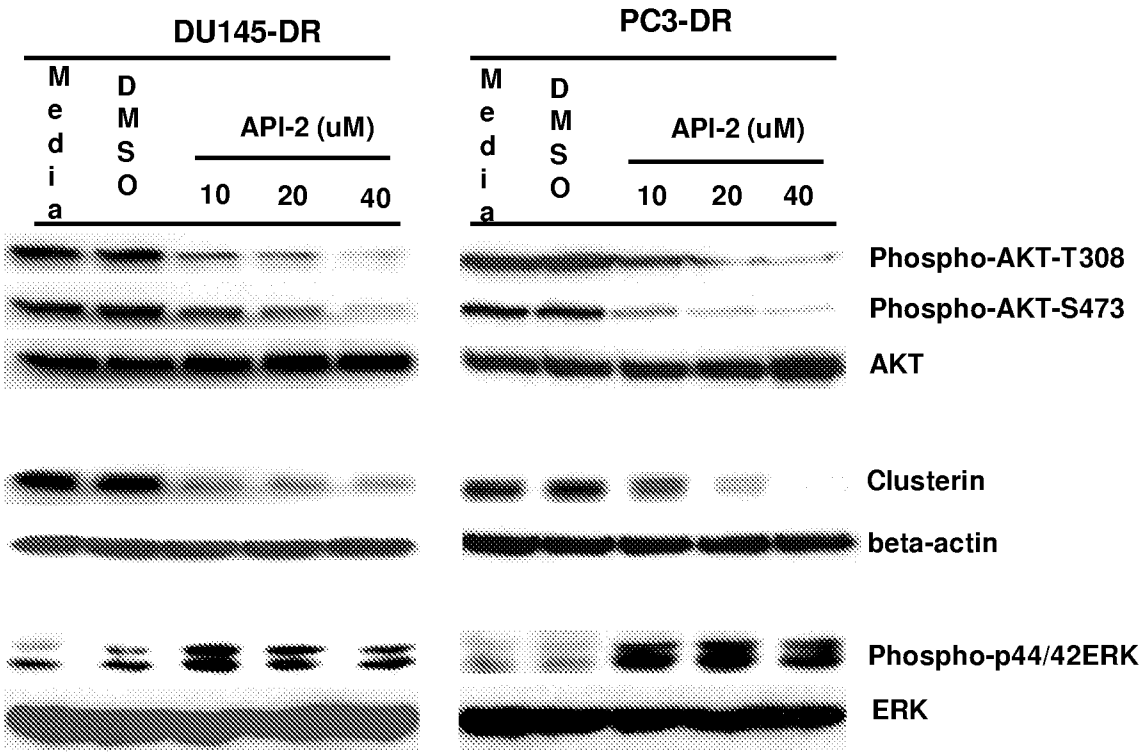

DU145-DR
PC3-DR
Media
DMSO
API-2 (uM)
10
20
40
Phospho-AKT-T308
Phospho-AKT-S473
AKT
Clusterin
beta-actin
Phospho-p44/42ERK
ERK

Figure 2B

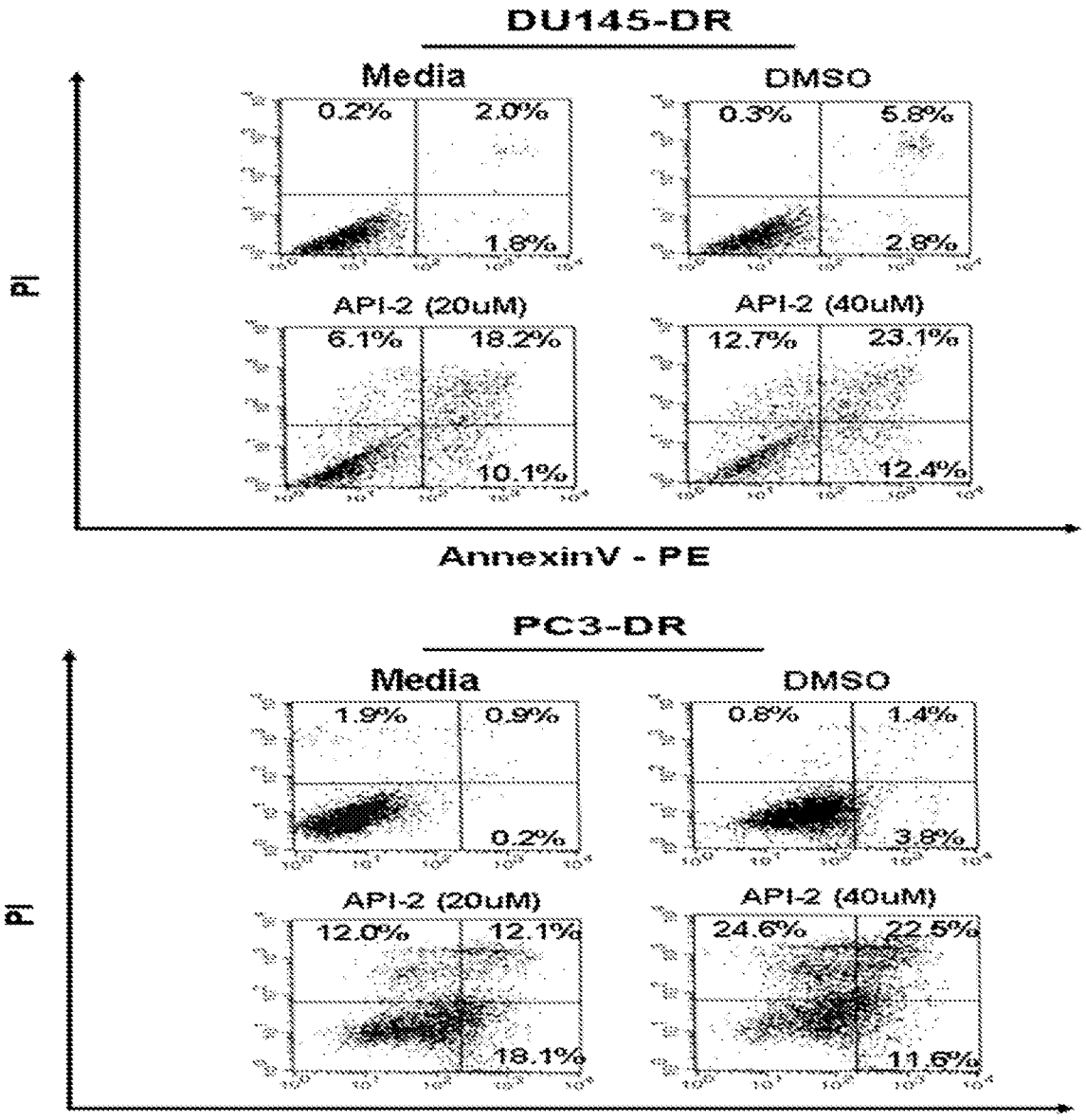

DU145-DR
Media
0.2%
2.0%
1.6%
DMSO
0.3%
5.8%
2.8%
API-2 (20uM)
6.1%
18.2%
10.1%
API-2 (40uM)
12.7%
23.1%
12.4%
PI
AnnexinV - PE
PC3-DR
Media
1.9%
0.9%
0.2%
DMSO
0.6%
1.4%
3.8%
API-2 (20uM)
12.0%
12.1%
18.1%
API-2 (40uM)
24.6%
22.5%
11.6%
PI

Figure 3A

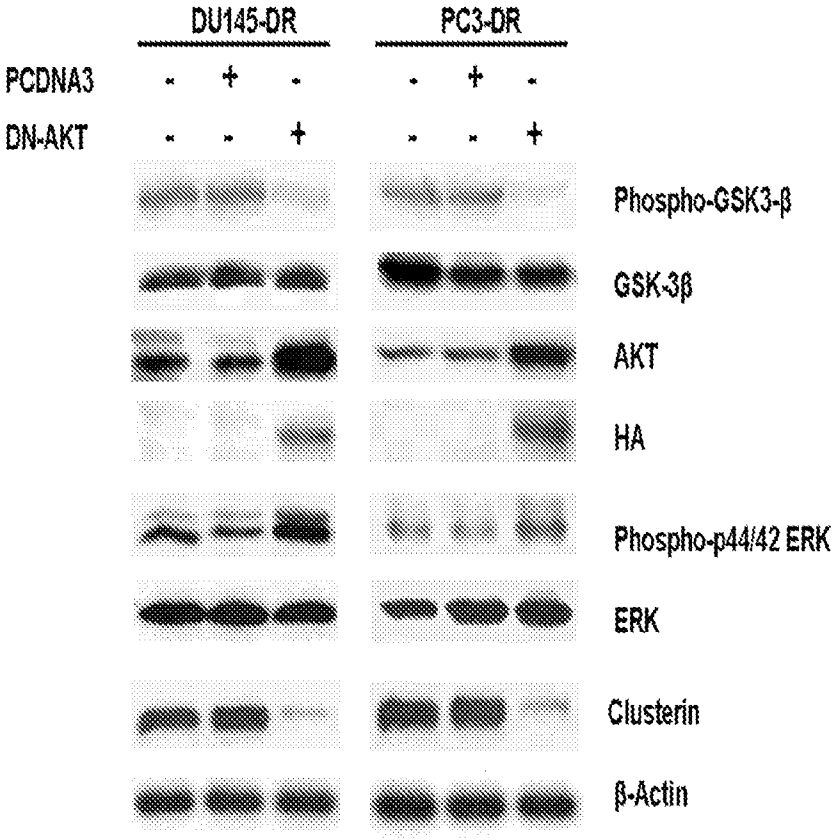


Figure 3B

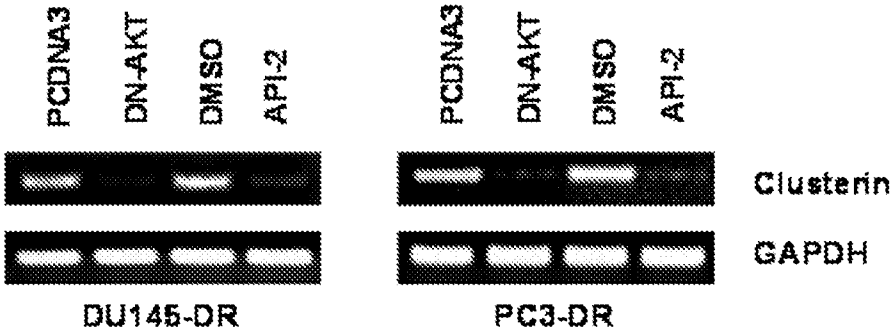


Figure 4A

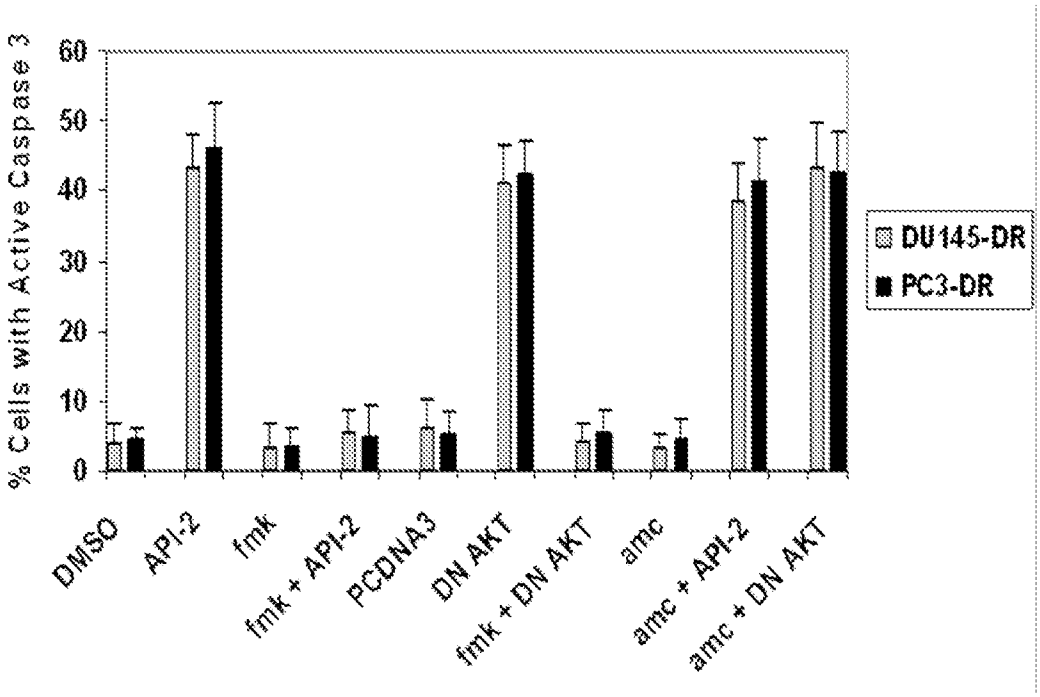


Figure 4B

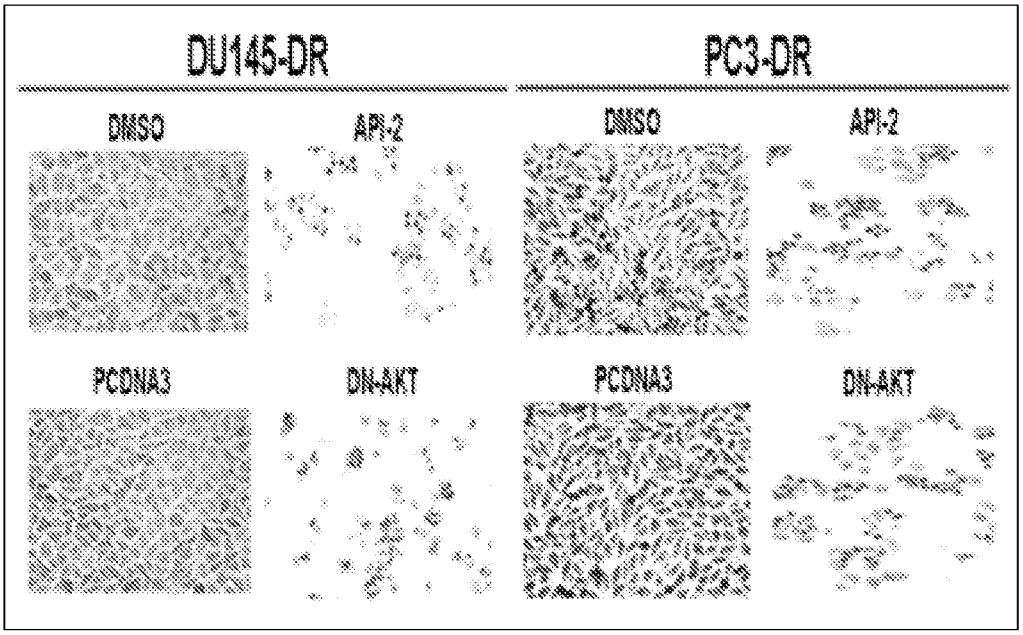


Figure 5A

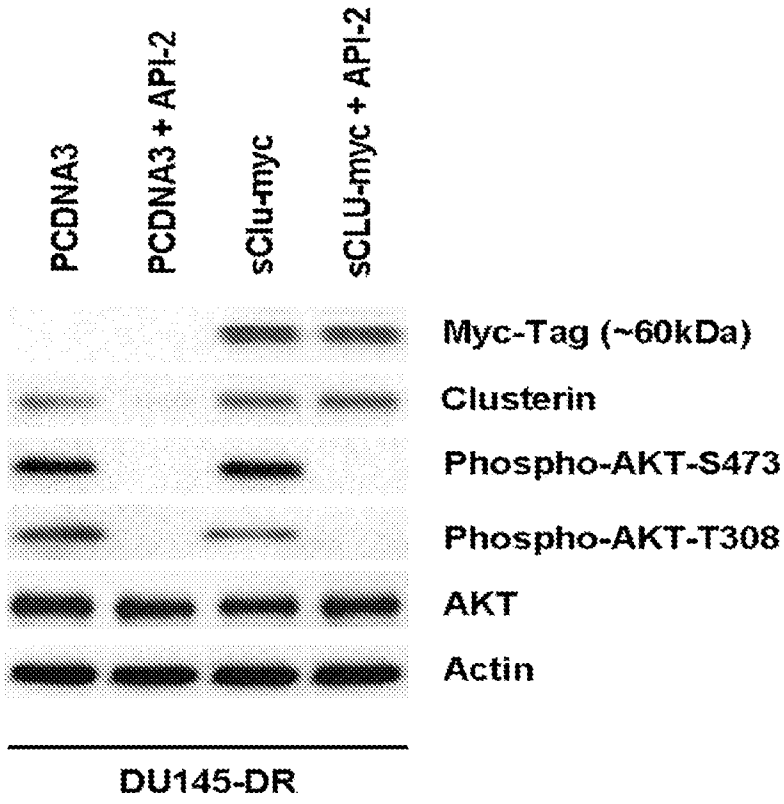

PCDNA3
PCDNA3 + API-2
sClu-myc
sCLU-myc + API-2
Myc-Tag (~60kDa)
Clusterin
Phospho-AKT-S473
Phospho-AKT-T308
AKT
Actin
DU145-DR

Figure 5B

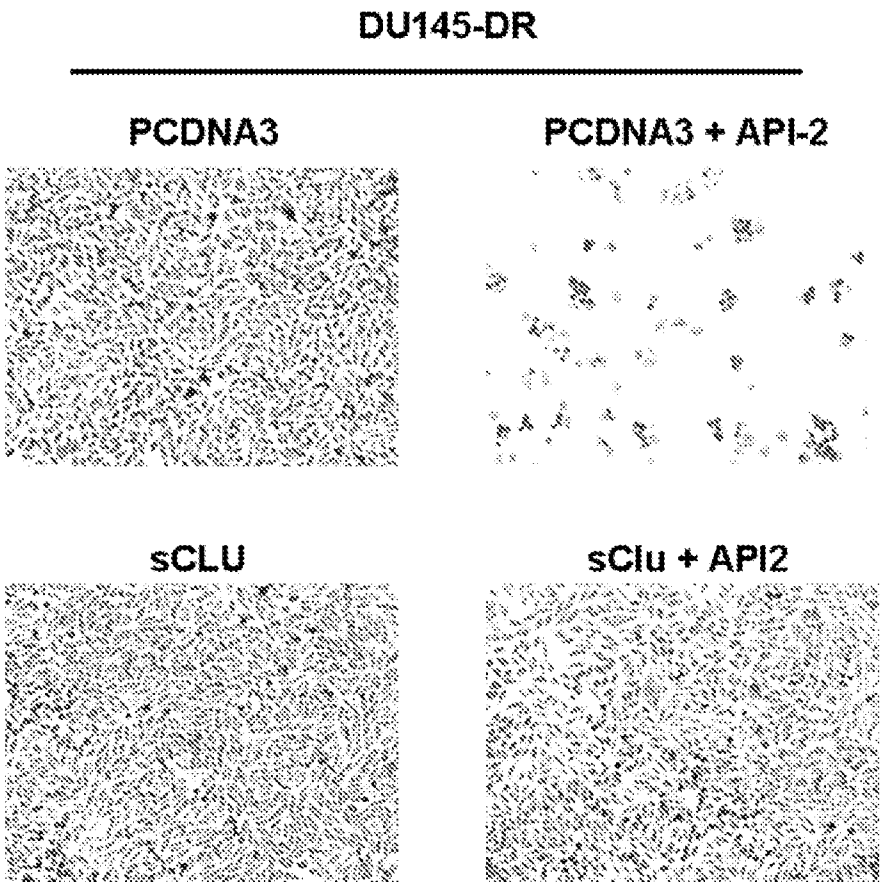


Figure 6

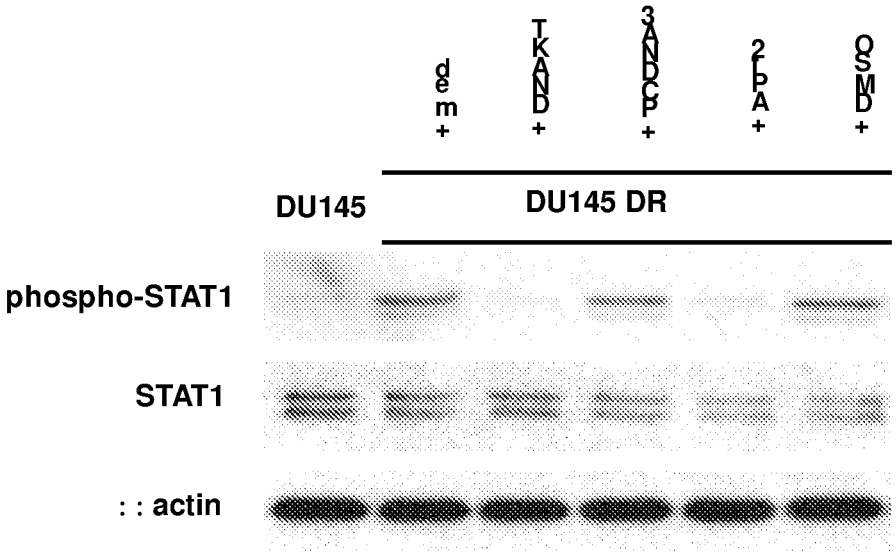


Figure 7A

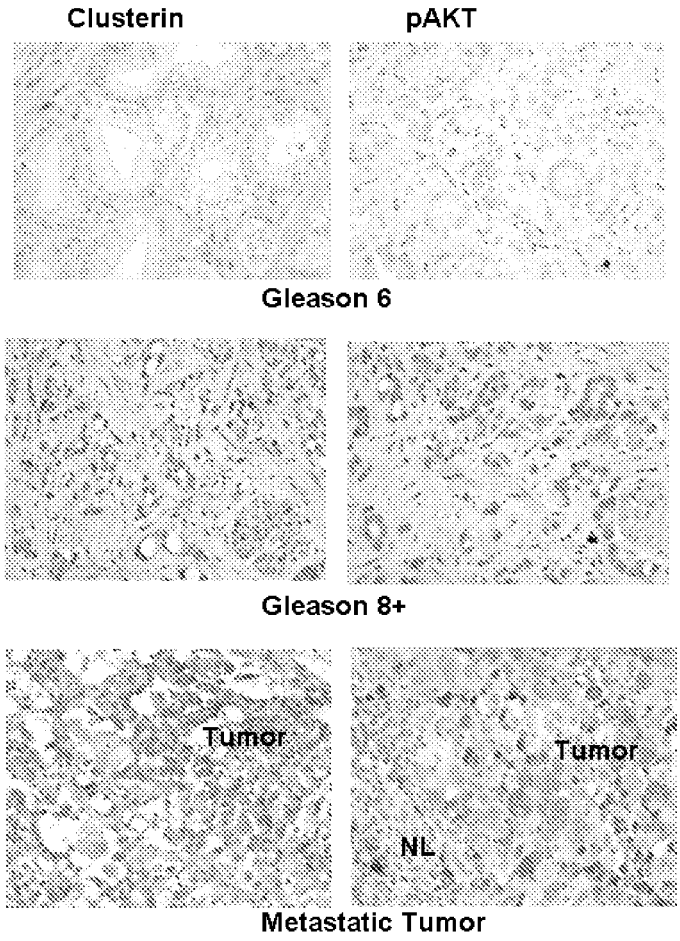


Figure 7B

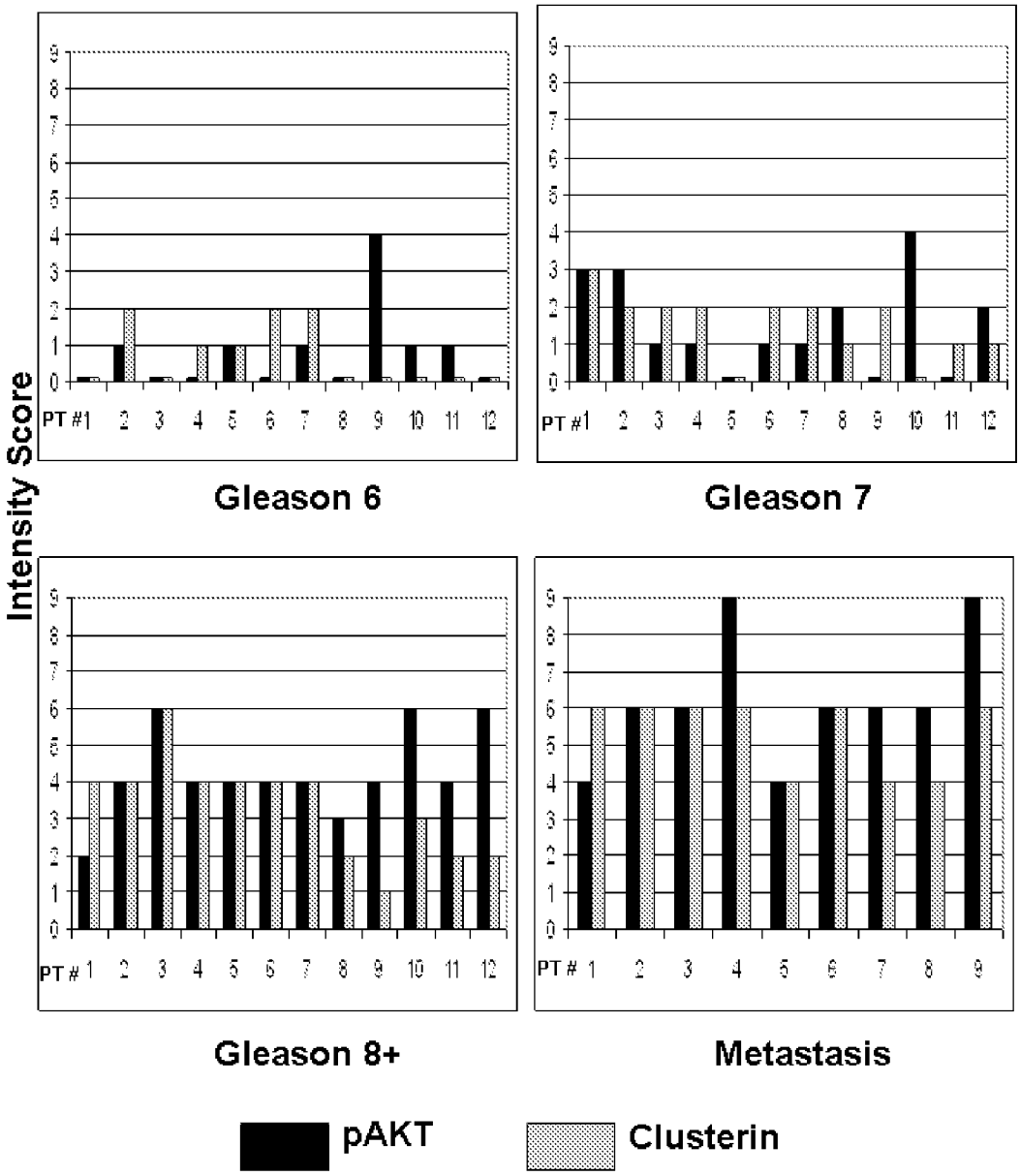
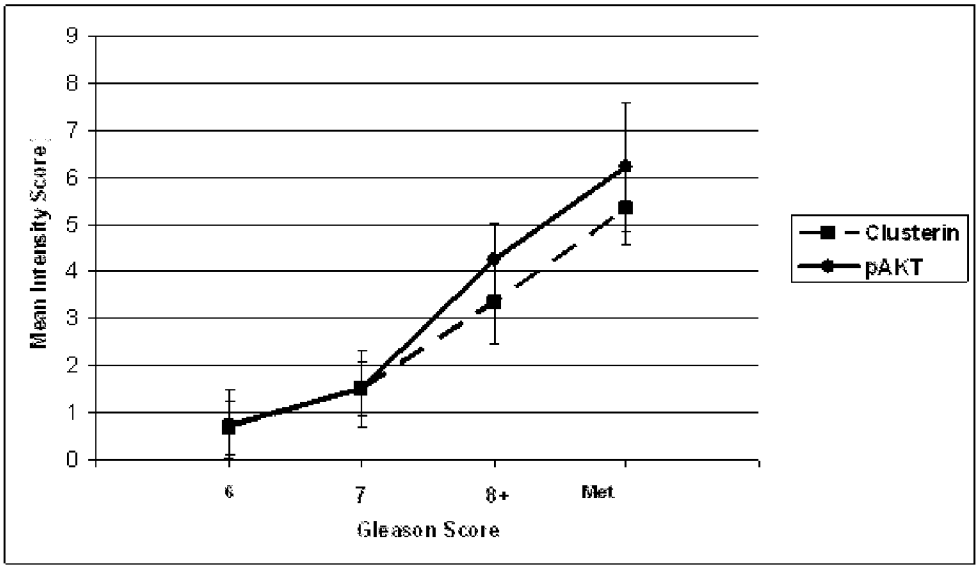

Intensity Score
PT #1 2 3 4 5 6 7 8 9 10 11 12
Gleason 6
Gleason 7
Gleason 8+
Metastasis
pAKT
Clusterin

Figure 7C


9
8
7
6
5
4
3
2
1
0
Mean Intensity Score
6
7
8+
Met
Gleason Score
Clusterin
pAKT