# Regulation of Androgen Receptor Function by Tyrosine Phosphorylation

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# ABSTRACT

MEHMET KARACA: Regulation of Androgen Receptor Function by Tyrosine Phosphorylation (Under the direction of Dr. Young E. Whang)

Androgen receptor (AR) re-activation under low androgen environment is the hallmark of castration resistant or hormone refractory prostate cancer (HRPC). Non-receptor tyrosine kinases phosphorylate AR in an androgen independent manner, and are thereby involved in HRPC. Herein, we investigated the role of Ack1 target phosphorylation sites (Tyr-267 and Tyr-363) on growth factor-regulated AR phosphorylation as well as AR transcriptional and functional activity. Both Tyr-267 and Tyr-363 have a critical role for ligand-dependent and –independent control of activity of AR. Treatment of LNCaP cells overexpressing full length or truncated AR (missing the ligand binding domain (LBD) with epidermal growth factor (EGF), heregulin, or Gas6 (ligand binding to Mer receptor tyrosine kinase and activating Ack1 downstream) induced AR phosphorylation at Tyr-267 and that phosphorylation was lost in the AR-Y267F mutant protein. The full length (FL) AR overexpressing cells proliferated strongly without androgen treatment and they reached optimal growth at a lower dose of DHT treatment (0.1 nM DHT). However, cells expressing full- or -truncated AR-Y267F mutant did not show androgen-independent proliferation and did not respond to androgen treatment. Overexpression of the Y267F mutant within full length- or truncated-AR showed significant reduction in soft agar colony formation, compared to the AR-WT. The extent of reduction in colony formation of

Y363F AR expressing cells was moderate, but not as much as Y267F. The analysis of AR subcellular localization by both immunoblotting and immunofluoresence assays suggested that mutating the Tyr-267 site impaired both androgen-dependent and –independent nuclear translocation, compared to the AR-WT. Global gene expression profiling analysis demonstrated that there were no common genes regulated by both full length AR and AR-Y267F, suggesting that mutating Tyr-267 has a significant impact on not only AR dependent target gene expression but also global gene expression of LNCaP cells. Taken together, the results of our study demonstrate that tyrosine kinase target phosphorylation sites are important for both ligand-dependent and ligand-independent activity of AR protein. Targeting upstream tyrosine kinases and the N terminal domain of AR in truncated AR missing LBD may expand the repertoire of therapeutics for combating prostate cancer.

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# TABLE OF CONTENTS

# LIST OF TABLES

Table

# LIST OF FIGURES

Figure

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AIB1 | Amplified in breast cancer 1 |
| Ack1 | Activated cdc42-associated tyrosine kinase 1 |
| ADT | Androgen deprivation therapy |
| AR | Androgen receptor |
| ARE | androgen response element |
| ARR-2PB | Androgen response region 2 probasin |
| BPH | Benign prostate hyperplasia |
| CGH | comparative genomic hybridization |
| COS-7 | Monkey kidney cells |
| CRIB | Cdc42/Rac interactive binding |
| CTD | COOH terminal domain |
| DHT | Dihydrotestosterone |
| EGF | Epidermal growth factor |
| EGFR | Epidermal growth factor receptor |
| FL-WT-AR | Full length wild type androgen receptor |
| FL-AR-Y267F | Full length Y267F AR |
| FL-AR-Y363F | Full length Y363F AR |
| Gas6 | ligand binding to Mer receptor tyrosine kinase |
| HER | Human epidermal growth factor receptor |
| HEK293 | Human embryonic kidney cells |
| hK2 | Human kallikrein2 |
| HRG | Heregulin |

| | |
|---|---|
| HRPC | Hormone refractory prostate cancer |
| HSP | Heat shock protein |
| LBD | Ligand binding domain |
| IGF-1 | Insulin growth factor 1 |
| IL-6 | Interleukin 6 |
| MAGE-11 | Melanoma antigen gene protein 11 |
| MAPK | Mitogen associated protein kinase |
| MMTV | Mouse mammary tumor virus |
| NLS | Nuclear localization signal |
| NTD | N-terminal domain |
| PCa | Prostate cancer |
| PIN | Prostatic intraepithelial neoplasia |
| PSA | Prostate specific antigen |
| PTK | protein tyrosine kinase |
| RTK | receptor tyrosine kinase |
| SH3 | Src homology 3 |
| SRC1 | steroid receptor co-activator 1 |
| TIF2 | Transcriptional intermediatory factor 2 |
| TR-WT-AR | Truncated wild type androgen receptor |
| TR-AR-Y267F | Truncated Y267F androgen receptor |
| TR-AR-Y363F | Truncated Y363F androgen receptor |

# CHAPTER I

INTRODUCTION

## 1.1 Prostate Cancer Epidemiology

Prostate cancer (PCa) is the most commonly diagnosed cancer (192,280 new cases per year) and second leading cause of death (27,360 deaths annually) among US men (Jemal et al., 2009). What causes prostate cancer is not completely known, but age, a family history, and African ancestry are strongly linked or associated with prostate cancer development (Gronberg, 2003; Schaid, 2004). The rate of prostate cancer is the highest among the African American men (AAM) in the United States (Brawley and Barnes, 2001) and mortality due to PCa in this group was higher than that of European American men (EAM) (American Cancer Society, 2007). The other ethnic groups such as Asian American men, American Indian and Alaskan Native men (AI/AN) and Hispanic men all have a lower prostate cancer and mortality rate compared to the EAM (American Cancer Society, 2007).

It is estimated that genetic predisposition to PCa accounts 9% of the PCa (Steinberg et al., 1990). Having a number of relatives that had PCa increases the risk of PCa. The estimated risk of getting PCa for a man with one first-degree relative, two relatives, and three relatives affected with PCa were 2.5, 5, and 11-fold, respectively. The recent research suggested that instead of one or two genes with a large effect determining a risk of getting prostate cancer, there may be many genes that may interact with environmental factors (Gronberg, 2003; Powell, 2007; Schaid, 2004). The results of linkage mapping studies indicated that there are a number of loci associated with the susceptibility to PCa. These loci are on chromosome 1, 8, 10, 16, 17, 20, and X (Schaid, 2004). The possible susceptibility to PCa candidate genes were HPC1 on chromosome 1q23-25, PCAP on chromosome 1q42-43, and CAPB on chromosome 1p36 (Schaid, 2004). In addition to these candidate genes, there are trinucleotide repeats encoding polyglutamine $(CAG)_n$ and polyglycine $(GGN)_n$ of

androgen receptor, whose polymorphism is associated with risk of PCa (Zeegers et al., 2004). In general, there is an association between shorter $(CAG)_n$ repeats and having more aggressive PCa, an earlier age of onset, and a longer time to recurrence, yet these effects appear to be population specific (Bratt et al., 1999; Giovannucci, 2002; Soronen et al., 2004) Although the rate of $(CAG)_n$ repeats were higher in AAM compared to EAM and Asians, that did not associate with an increased PCa risk (Gilligan et al., 2004; Powell et al., 2005).

In addition to genetic linkage studies, the clinically more relevant genetic polymorphism studies have identified that a polymorphism in CYP17 might be associated with susceptibility to prostate cancer in AAM, but not in men of European descent (Ntais et al., 2003). In another study, the proportion of G allele of CYP3A4 was found to be higher in AAM compared to EAM (Powell et al., 2004) and later in a case-control study, a strong association between the variant G allele and aggressive PCa in AAM were reported (Bangsi et al., 2006; Rebbeck et al., 1998).

There is mounting evidence that dietary (environmental) and life style factors play a critical role in the pathogenesis of PCa. There is a striking difference in distribution of PCa incidence and mortality rates in different geographical parts of the world (American Cancer Society, 2007). Countries like Japan and China have historical lower rates of PCa and mortality compared to that of the United States and Caribbean nations (Liu et al., 2001; Schaid, 2004). The high intake of saturated fat, red meat, and dairy products was found to be associated with the pathogenesis of PCa (Gronberg, 2003; Schaid, 2004). The rate of animal protein in total protein consumed was higher in the US than in Asian countries. This may account for over 20-fold increase in PCa rate in the US (Griffiths et al., 1999; Wang et al., 2005). Although conclusive evidence for the association of obesity and the development of

prostate cancer has not been found yet, there are reports that suggest the risk of getting aggressive cancer increases with obesity (Buschemeyer and Freedland, 2007).

## 1.2 Natural History of Prostate Cancer

Prostate cancer is a gland specific cancer that affects the prostate gland. However, other glands in the urogenital tract such as cowper gland have no known cancer. Anatomically, the prostate gland is composed of three regions, peripheral, central and transition zone. Unlike benign prostate hyperplasia (BHP), prostate cancer occurs in peripheral zone (McNeal et al., 1988). There are two types of cells that make up the epithelia of prostate, basal and luminal epithelial cells. Basal cells, not expressing AR, contain stem cells and trans-amplifying (TA) cells that have limited proliferative capacity and differentiated from basal cells. The terminally differentiated secretory luminal cells expressing AR result from cell division, differentiation, and migration of TA from the basal to luminal layer (Litvinov et al., 2003). The surrounding stromal cells that are AR positive are the source of growth and survival factors in the paracrine regulation of the glandular epithelium. In the normal prostate, in response to stromal cell signaling, the ligand bound AR shows a growth-suppressing effect and mediates the secretory protein (PSA and HK2) production. The anti-proliferative effect of AR was in response to the paracrine signaling of stromal cells, yet in prostate cancer, this paracrine signaling evolved into autocrine signaling with self sufficiency (Gao et al., 2001).

From initiation to development of prostate cancer, there are several steps. Like any other cancer, mutation in normal prostate epithelium and accumulation of other somatic mutations underlines the morphological changes that occur with tumor development. The natural history of prostate cancer begins with normal prostate exposed to proliferative

inflammatory atrophy (PIA), followed by prostatic intraepithelial neoplasia (PIN) that is a precursor for invasive adenocarcinoma, and eventually to prostatic adenocarcinoma (Nelson et al., 2003b) (Nelson et al., 2003; Shen-Abate and Shen 2000). After cancer develops, it may eventually metastasize into secondary distant sites such as bone and lymph node (Abate-Shen and Shen, 2000; Litvinov et al., 2003; Nelson et al., 2003b). There are several lines of evidence that suggest that AR expression is sustained at every stage of prostate cancer (primary and metastatic), as well as hormone refractory prostate cancer (HRPC) (Taplin, 2008). AR knockdown results in inhibition of growth and induction of apoptosis in both prostate cancer cells and tumor xenografts (Taplin and Balk, 2004). There is additional evidence that the AR signaling pathway exhibits sustained activity during prostate cancer initiation, development and progression to the hormone refractory disease. (1) The AR gene amplification and AR protein expression (Taplin et al., 1999). (2)The AR overexpression was the only detectable change occurring in castration recurrent isogenic cell lines derived from the xenograft tumors that are resistant to the androgen deprivation therapy (Chen et al., 2004). (3) The AR protein was active and stabilized in the CWR22R prostate cancer cell line derived from the castration recurrent prostate cancer that render CWR22R to be induced by lower levels of androgen (Gregory et al., 2001). (4) Even in castration recurrent prostate cancer cells, knocking down of AR abrogated cell proliferation and induced apoptosis suggesting that AR has a critical role in the castration recurrent stage of disease.

## 1.3 Prostate Cancer Therapy

Androgen receptor is not only involved in normal prostate development, but also in prostate cancer development and progression. In 1941, Huggins and Hodges's discovery of the dependence of prostate cancer to androgens led to androgen deprivation therapy (ADT)

as the first line of treatment against metastatic prostate cancer (Huggins and Hodges, 2002). ADT is carried out by using surgical or medical castration. ADT does not lead to complete androgen depletion, since the adrenal cortex produces approximately 5-10% of total androgen produced in the body. The majority of the patients respond to ADT positively with tumor shrinkage. This is temporary as they progress with tumor growth and relapse even under the low androgen environment with a median duration of 18-24 months in patients with metastasis. Cancer in this stage called hormone refractory (HRPC) or castration resistant prostate cancer (CRPC). The median survival of men with HRPC is about 1.5 years and chemotherapy is the mainstay of therapy in this stage of disease (Smaletz et al., 2002).

The multifocal and heterogeneous nature of PCa makes its diagnosis and management of PCa a very complex process. Within one tumor, it is not uncommon to detect simultaneously multiple clones of tumor foci. These heterogeneous foci might have different degrees of genetic alterations and thereby progress with different degrees of aggressiveness. The presence of multifocal prostate cancer at diagnosis ranged from 60-90% (Andreoiu and Cheng, 2010). Although the biological basis of multifocal and histological heterogeneity of prostate cancer has not been explained completely, there are two main theories that attempt to explain this phenomenon: (1) multiclonality of the initial diseases, and (2) clone evolution from the initial disease. Although the multifocal and histologically heterogeneous nature of prostate cancer has complicated the treatment choices, recently there have been numerous attempts to devise novel approaches to reduce the complications of radical resection of prostate cancer as well as complete eradication of local lesions with good survival outcome (Barqawi and Crawford, 2005; Koch et al., 2007; Polascik and Mouraviev, 2009).

Anatomically, prostate cancers have originated most commonly at peripheral zone (PZ) and transition zone (TZ) (McNeal, 1968, 1969, 1981a, b, 1984, 1988; McNeal et al., 1988; McNeal et al., 1986). These initial studies demonstrated that 70% of tumors take place in the PZ, while 25% originated in TZ. The tumors rising in PZ can commonly be detectable with transrectal palpation and biopsy. However, tumors in TZ cannot be easily detectable with biopsy and are mostly discovered with TURP specimens (Augustin et al., 2003). Patients with TZ tumors had an 81% 5-year cure rate compared to 53% seen in PZ tumors (Stamey et al., 1998). TMPRSS2-ERG gene fusion was commonly detected in PZ cancers and not in TZ cancers (Guo et al., 2009).

Cryosurgery, brachtherapy, high intensity focused ultrasound, external beam radiation therapy (EBRT), high-intensity focused ultrasound radio frequency, interstitial ablation, and others have been used to treat locally grown prostate cancer. The limitations in preoperative mapping of all tumor lesions that intrinsically multifocal and histological heterogeneous have a significant impact on selecting patients for focal treatment as well as overall determination of optimal surgical management. The selection of treatment plans is made with taking into account both tumor related factors (i.e. clinical stage, PSA, and Gleason score) and patient related factors (i.e. age, other illness, and patient preference). Radical prostatectomy (RP) is a treatment of choice when the patient is young (<70 years old) with organ confined disease (Bill-Axelson et al., 2005; Gettman and Blute, 2010). The success of RP depends on complete removal of tumor. Preoperative staging and tumor characteristics are instrumental in identifying the region that contains tumor. The patients in the low risk group defined as clinical T1 and T2 disease, PSA$\leq$ 10 ng/ml and Gleason score $\leq$ 6 have a lower probability of cancer recurrence compared to the patients in the high risk groups. External beam radiation

therapy (EBRT) is an advancement of three-dimensional conformal radiotherapy (3D-CRT) with the use of high energy photon field in precisely defined locations in the prostate by using computer generated tomographic images of patient's anatomy (Ling et al., 1996; Perez et al., 2000; Potters et al., 2000). Depending on the extent of tumor involvement, prostate and part of seminal vesicles are irradiated. The typical treatment of EBRT takes 5 days a week with 1.8-2 Gy daily in a 10-20 minutes per session to a total dose of 70-78 Gy (Horwitz et al., 2001). In brachytherapy (seed implant therapy), the radioactive sources by itself are placed into tumors (D'Amico and Coleman, 1996). Depending on the characteristics of cancer and patient, low-dose rate or high-dose rate (HRT) is recommended. There are 3 energy sources used for this therapy: iodine-125, palladium-103 for LDR and iridium-192 for HDR (Stock and Stone, 2010).

The current standard of care for metastatic HRPC is docetaxel treatment. The current rate of survival in HRPC patient in response to standard treatment modalities shows that there is a space for improvement in novel therapeutic approaches. These approaches includes angiogenesis inhibitors, small molecule inhibitors, novel cytotoxic therapeutics, anti-sense oligonucleotide based targeted therapeutics (Richard et al., 2006).

Metastasis to bone is a common event in advanced prostate cancer and can be a cause of great pain and skeletal complications. Furthermore, long-term ADT will enhance the loss of bone mineral density in patients (Toni et al., 2010). Bisphosphonates are used to reverse the aforementioned osteoclastic bone destruction. In addition to its affect on bone destruction, bisphosphonates may be effective to inhibit bone metastasis by modulating matrix metalloproteinases (MMPs), cell adhesion, and cancer cell growth (Lee et al., 2001). Because of its beneficial affect on skeletal complications, FDA approved zoledronic acid for

use in patients with HRPC who have not respond to at least one regimen of hormonal therapy (Saad et al., 2004).

Prostate cancer generally has a long natural history with slow progression. Therefore, this long time span may enable induction and activation of T-cell mediated antitumor activity (Florian et al., 2007). Second, prostate is a target of chronic inflammation that can be associated with autoimmunity or pathogenic infection (Bardia et al., 2009; Florian et al., 2007). Since these patients have the capacity to enhance their response to these non-malignant pathologic conditions, it is expected that these patients may mount cell-mediated immune response that may suppress the prostate tumor progression. Unlike other vital organs, prostate is a dispensable organ and autoimmunity due to immune therapy will have a very limited consequence to the patient. There has been intensive work to develop vaccine by using dendritic cells, APC8015 (Provenge, Dendreon, Seattle, WA) or GVAX (whole cell vaccine) and Prostvac-VF (viral vaccines) (Stavridi et al., 2010). APC8015 is an autologous vaccine developed by harvesting $CD54^{+}$ dendritic cells and combined with the recombinant fusion protein PAP (prostate acid phosphatase) and the cytokine GM-CSF. GVAX (Cell Genesys, San Fransisco, CA) is composed of a combination of two prostate carcinoma cell lines (PC3 and LNCaP) that have been genetically modified to secrete GM-CSF.

The enhanced understanding of the molecular basis of cancer progression and therapeutic resistance has generated therapeutic targets that cannot be inhibited by small molecule or antibody inhibition. Therefore, antisense oligonucleotide (ASO) may be a perfect tool to prevent cancer progression. For example, the anti-apoptotic Bcl-2 gene was overexpressed in therapy resistant prostate cancer and targeting this gene may improve the chemotherapy induced apoptosis (Gleave et al., 2005). Because of its role in therapy

resistance, ASO targeting first six codon of Bcl-2 has been developed (G3139). There have been a number of clinical trials in HPRC combining cytotoxic chemotherapy with G3139. The results of phase I trials using G3139 and either mitoxantrone or docetaxel (Chi et al., 2001; Tolcher et al., 2004) showed that addition of G3139 to standard chemotherapy regimens did not increase toxicity.

Another successful use of a second generation antisense inhibitor was reported to inhibit the clusterin gene, also known as testosterone repressed message-2 (TRPM-2). It has a chaperon function to stabilize the protein conformation in cells under stress by preventing protein aggregation and prevention. In prostate cancer, the clusterin level has been correlated with the pathological grade (Steinberg et al., 1997). Furthermore, the clusterin level was significantly elevated in response to neoadjuvant hormone therapy (July et al., 2002). Preclinical studies indicated that clusterin prevented apoptotic cell death due to androgen withdrawal, chemotherapy, and radiation (Miyake et al., 2000a; Miyake et al., 2000b; Zellweger et al., 2001). The results from preclinical models of prostate cancer indicate that OGX-11, the clusterin antisense inhibitor enhanced the efficacy of androgen withdrawal, chemotherapy, and radiation by reducing the expression of clusterin and concomitantly enhancing the apoptotic response (Miyake et al., 2000a; Miyake et al., 2000b; Zellweger et al., 2002; Zellweger et al., 2001)

The multiple roles of endothelin and its receptor ET-A on cellular physiology and paracrine signaling in prostate cancer made it a therapeutic target. Endothelin by interacting with the MAPK pathway influences the cell growth and regulates apoptosis through its interaction with Bcl-2 and PI3K/Akt pathways (Nelson et al., 2003a). Atrasentan (Xinlay, Abbott Labs, Abbott Park, IL) is a potent ET-A receptor antagonist developed and used in

clinical trials. Although the result of the phase III trial is not satisfactory (in time to progression), its role in combination therapy remains to be determined.

Angiogenesis is an important part of cell growth, invasion and metastatic potentials of solid tumors (Ferrara and Kerbel, 2005). Therefore, agents targeting angiogenesis may have a potential impact to delay progression or to induce regression of tumors if used in combination with other treatment modalities (Kluetz et al., 2010). Bevacizumab is a humanized murine monoclonal antibody that neutralizes vascular endothelial growth factor (VEGF) activity and has shown potential in HRPC (Aragon-Ching and Dahut, 2008). The sustainable tumor growth requires angiogenesis, the development of new blood vessels from the pre-existing vascular bed. The critical balance between endogenous angiogenesis inducers (fibroblast growth factor, VEGF) and inhibitors (endostatin and angiostatin) of endothelial cells is important for cell proliferation and invasion. Likewise, thalidomide inhibits b-FGF induced angiogenesis. In phase II trials, treatment of HRPC patients with thalidomide resulted in 18% patients having a PSA decline of $\geq 50$ and 27% of patients having a PSA decline of ≥40% (Figg et al. 2001). Another phase II clinical trial used thalidomide in combination with docetaxel reported greater PSA decline (51% versus 37% demonstrating >50% PSA decline) as well as median progression free survival (5.9 months versus 3.7 months) and improved survival (29.8 moths in the combination group compared with 14.7 months in the docetaxel only group).

## 1.4 Androgen Receptor Genomic Structure

Androgens are important for male sexual differentiation and development as well as development of prostate cancer. Androgens exert their effects through androgen receptor. Testosterone and dihydrotestosterone (DHT) are the physiological ligands for the AR. DHT

is synthesized from testosterone by 5α-reductase enzymes. The human AR gene, located on chromosome Xq11-12, consists of 8 known exons which encode ~110 kDa protein. The AR protein shows typical modular functional units of the steroid hormone receptor family of transcription factors (Bain D. L., 2006). The AR shows homology to the genetic structure of other steroid hormone receptor such as the estrogen, progesterone, glucocorticoid, retinoid, mineralocorticoid and thyroid hormone receptors (Matias et al., 2000; Rozanov et al., 2004; Shaffer et al., 2007). Like other steroid receptors, AR has three functional domains: an amino terminal domain, a central DNA-binding domain, and a carboxy ligand binding domain. A large part of AR protein, almost 60%, is encoded by exon 1 and designated as the $NH_2$-terminal domain (NTD) which contains a ligand-independent AF1 transcriptional regulatory domain (Figure 1.1). AR has two transcriptional activation function domains (AF-1 and AF-2). Unlike AF-1, AF-2 is a ligand-dependent coactivator binding domain which shows great homology among steroid receptors. Transcriptional activity of the C-terminal domain (CTD) of AR was very weak compared to the other steroid hormones. AR NTD has no sequence homology to the other receptors and show strong transcriptional activity (Dehm and Tindall, 2006b, 2007; Rozanov et al., 2004). The exon 2 and 3 encode the central DNA binding domain (DBD) with two zinc finger motifs (Figure 1.2). The rest of the exons (4 to 8) encode ligand binding domain (LBD) where the ligand-dependent AF-2 activity resides.

The subcellular dynamics of AR is orchestrated by ligand binding. AR without a ligand is sequestered in the cytoplasm by the heat shock protein family of chaperons. Androgen binding to the LBD of AR induces conformational changes and stabilizes AR. This leads to its dissociation from heat shock proteins and translocation into the nucleus. Within the nucleus, AR, an activated transcription factor, binds to the sequence motif called

androgen response elements (AREs, CGTACAnnnTGTTCT) in the promoter and enhancer regions of the genes. Once AR bounds to AREs, it coordinates the recruitment of cofactors as well as chromosomal remodeling proteins that are essential for the transcriptional activation (Alimirah et al., 2006; Shang et al., 2002). In addition to its function as a transcription factor, AR involved in non-genomic androgen effects, intracellular calcium flux and kinase activation (Heinlein and Chang, 2002).

## 1.5 Role of Androgen Receptor in Hormone Refractory Prostate Cancer

Androgens are essential for normal development of sexual characteristics as well as normal prostate growth and development. The androgen dependency of normal prostate extents into the initial stages of prostate cancer. However, PCa eventually becomes "androgen independent" or castration resistant under the selective pressure of androgen deprivation therapy (ADT), which is the mainstay of treatment of advanced prostate cancer devised by Huggins and Hodges in 1941. Although PCa becomes castration resistant, AR and its downstream signaling pathways are found to be active in the hormone refractory stage of PCa. There are several explanations of how AR becomes reactivated under the low androgen microenvironment (Borchert et al., 2007; Koivisto, 1997; Koivisto et al., 1995; Koivisto et al., 1996; Litvinov et al., 2003; Liu et al., 2005; Pienta and Smith, 2005; Williams et al., 1996). These include AR gene amplification, post-transcriptional modification of AR protein, growth factor-regulated activation of AR, mutations in the ligand binding domains, and differential expression of AR associated co-regulatory proteins (i.e. enhanced interaction of AR with coactivators). AR gene amplification leads to AR protein overexpression, thereby keeping the AR signaling pathway intact even in the absence of the normal level of androgens. AR gene amplification accounts for the one-third of tumors (Koivisto et al.,

1997; Koivisto et al., 1996; Linja et al., 2001). The mutations in LBD of AR explain approximately 10% of androgen independent prostate cancers (Taplin et al., 2003). AR post-transcriptional modifications such as phosphorylation may enhance the AR protein stability. Growth factors such as EGF, heregulin may stimulate the phosphorylation and activation of AR. The anti-androgen drugs used in combined androgen deprivation therapy induce mutations in the ligand binding domain of AR that broadens the ligand specificity of AR. The frequency of AR mutations are higher in metastases than local diseases and these mutations may allow AR to bind with estrogens, glucocorticoids and anti-androgens (Culig et al., 2003; Marcelli et al., 2000). For example; bicalutamide induces mutations in LBD of AR that can turn this strong antagonist into an agonist of AR. There might also be protein kinases that can directly phosphorylate and activate AR. As an alternative to the aforementioned mechanisms of aberrant AR activation, the measurement of androgen levels in prostate cancer specimens indicated that the tissue androgen levels under androgen deprivation therapy are still sufficient to activate AR (Mohler et al., 2004; Titus et al., 2005).

The newly found mutations made the AR not only sensitized to lower levels of androgens and dehydro epiandrosterone (DHEA) but also broaden the ligand choices such as 17β-Estradiaol, progesterone, corticosteroids, and anti-androgens (Chen et al., 2005; Monge et al., 2006; Shi et al., 2007; Yeh et al., 1998). There are several reports that showed AR nonsense mutation arose and resulted in truncated AR protein which lacks ligand binding domain and is constitutively active (Alvarado et al., 2005; Lapouge et al., 2007). These nonsense mutations were detected in metastatic tumors and sometimes coexist with other AR mutants such as T877A mutation. Alvarado et al. (2005) reported that a particular non-sense AR mutant Q640X was responsible for the androgen independent transcriptional activity

observed with T877A. The studies with TRAMP model suggested that somatic mutations were adaptive responses to ADT since the rate of mutation increases dramatically when TRAMP mice switched to ADT (Han et al., 2001). The mutation analysis of human prostate cancer shows parallelism to the mouse model in that somatic mutations were more commonly detected in castration recurrent disease (Linja and Visakorpi, 2004). In summary, these gain-of-function somatic mutations may provide prostate cancer a growth advantage under the low androgen environment and render AR as a proto-oncogene.

## 1.6 Role of N-Terminal Domain of AR in Hormone Refractory Progression

The AR NTD domain may play a significant role in mediating prostate cancer therapy resistance (Dehm and Tindall, 2006b, 2007). It has been instrumental to study the structure function relationship of NTD to characterize the NTD domain which cannot be defined due to the amorphous nature of the NTD domain. There are co-activators that directly bind and activate NTD such as members of p160 family (Alen et al., 1999; Bevan et al., 1999). Steroid receptor co-activator 1 (SRC1) binding to AR does not require LxxLL motif in the AF-2 domain to bind and activate AR (Bevan et al., 1999). Likewise, the X-chromosome linked melanoma antigen gene product, MAGE11, binds to FxxLF motif in NTD and that stabilizes AR protein and enhances its transcriptional activity (Figure 1.2) (Bai et al., 2005). Furthermore, MAGE-11 binding to FxxFL motif enhances AF-2 accessibility to LxxLL containing the p160 coactivator such as TIF-2 (Wilson, 2007).

The expansion of the NTD region with increased AF-1 activity relative to AF-2 has been evolutionarily observed for AR and other nuclear receptors (Rozanov et al., 2004). The majority of transcriptional activity of AR has been mediated by the NTD AF-1 domain. The AF-1 consists of two functional subunits: transactivation unit 1 (TAU1) and TAU5 (Jenster,

1999). Further deletion studies showed that TAU1 has two transcriptionally distinct motifs (AF-1a and AF-1b). The core motif that mediates transcriptional activity of TAU1 was found to be $^{178}$LKDIL$^{182}$ (Figure 1.2) (Sadar, 1999). The TAU5 motif ($^{435}$WXXLF$^{439}$) was found to be direct binding sites of SRC1 and protein kinase C related kinase-1 coactivator proteins (Callewaert et al., 2006; Metzger et al., 2003).

The reports indicated that prostate cancer evades the hormone ablation therapy and becomes HRPC due to ligand independent transcriptional activity (Dehm and Tindall, 2006a, 2007). Dehm et al. (2008) hypothesized that selective pressure due to hormone ablation therapy leads to activation of NTD where AF-1 resides (Dehm et al., 2008). The deletion of LBD generates an androgen independent transcriptional activator protein(Jenster, 2000). Libertini et al. (2007) showed that calpain mediated AR cleavage created an AR protein variant that lacks CTD and is constitutively active (Libertini et al., 2007). Their *in vitro* and *in vivo* characterization of the mechanism of calpain mediated AR activation suggested that calpain cleavage of AR led to constitutively active AR which was more robust in transactivating transcription from the PSA promoter. Furthermore, they reported elevated expression of the truncated AR isoforms in some prostate tumor tissues. The Rv1 cells used in this study has been derived from CWR22 cells and expresses concomitantly both truncated AR isoforms and full length wild type AR. The treatment of Rv1 cells with a calpain inhibitor or HIV protease inhibitors in the absence of androgens induced apoptosis by preventing the generation of constitutively active truncated AR, suggesting that full length wild type AR drives cells into apoptosis in the absence of androgens. This study underscored the importance of a mechanism that led to constitutive activation of AR in the absence of androgen; thereby a strategy to prevent calpain mediated activation of AR in the hormone

refractory stage of prostate cancer may be an option to add to the repertoire of limited therapeutic choices.

Recent report underscored the importance of somatic mutations arising under ADT to evade the selective pressure of therapy. Hu et al. (2009) using *in silico* sequence analysis of the AR gene identified 7 cryptic exons (AR variants) that harbor premature stop codons downstream of DBD, generating AR-LBD truncated proteins when they have been translated (Hu et al., 2009). From these 7 variants, they further characterized two of them (V1 and V7) and developed an antibody that specifically detects AR-V7. There is a significantly elevated expression (~20 fold) of AR-V1 and AR-V7 mRNA in HRPC tumor samples compared to the hormone naïve prostate cancer. The elevated expression of the AR-V7 splicing variant in a subset of hormone naïve prostate cancer was predictive for recurrence of prostate cancer after surgical treatment (Hu et al., 2009). AR-V7 was constitutively active and localized in the nucleus in the absence of androgens. Furthermore, androgen stimulation did not change the proportion of AR-V7 localized in the nucleus. Functional assays done by transfecting AR-V7 into PC3 cells showed that AR-V7 localized to the nucleus and induced PSA reporter activity in an androgen independent manner. Microarray gene expression analysis of LNCaP cells transfected with AR-V7 showed that AR-V7 stimulated the expressions of androgen dependent genes KLK3, KLK2, NKX3-1, FKBP5, and TMPRSS2 in the absence of androgens. Mutation induced truncated AR missing LBD has been reported as constitutively active (Ceraline et al., 2003). Ceraline et al. (2003) using a yeast based functional assay showed that AR-Q640Stop/T877A mutation isolated from bone marrow metastasis of hormone refractory prostate cancer was constitutively active in the absence of androgens. They indicated that elevated transcriptional activity observed with the luciferase reporter

assay was not affected by the presence of DHT treatment. This report shows that mutations in androgen receptor can be a basis for transition of prostate cancer from hormone dependent to independent.

The recent studies elucidated the mechanism of alternative splice variants of AR that generated a ligand-independent constitutively active AR (Dehm et al., 2008; Dehm and Tindall, 2006a). Dehm et al. (2008) reported that the 22Rv1 cell line, derived from the relapsed CWR22 xenograft during androgen ablation therapy, expressed three isoforms of AR: a full length version with duplicated exon 3 ($AR^{ex3dup}$), and two truncated versions lacking the COOH terminal domain (CTD) ($AR^{ex1/2/2b}$ or $AR^{ex1/2/3/2b}$) (Dehm et al., 2008). Unlike the reports of Tepper et al. (2002) and Libertini et al. (2007), Dehm et al. showed that truncated isoforms ($AR^{ex1/2/2b}$ or $AR^{ex1/2/3/2b}$) of AR were not due to proteolytic cleavage of AR with exon 3 duplication ($AR^{ex3dup}$) but derived from distinct mRNA species. AR NTD isoforms expressed in 22Rv1 cells are the result of the splicing of a novel exon 2b after AR exon 2 ($AR^{ex1/2/2b}$) or exon 3 ($AR^{ex1/2/3/2b}$) (Dehm et al., 2008). Exon 2 encodes the first zinc finger that harbors the recognition helix that directly engages with one hexameric half site with an androgen response element (ARE) (Shaffer et al., 2007). Based on this structural information, $AR^{ex1/2/2b}$ has only one zinc finger and missing second canonical zinc finger that is encoded by exon 3 and mediates dimerization with an AR molecule engaged with the neighboring ARE half site (Shaffer et al., 2007). siRNA functional analysis of these isoforms showed that siRNA targeting exon 1 abolished the protein expression of both $AR^{ex3dup}$ and truncated isoforms ($AR^{ex1/2/2b}$ or $AR^{ex1/2/3/2b}$). However, siRNA targeting exon 7 specifically abolishes the full length AR protein expression but not the truncated AR protein that runs around 75 kDa. The $AR^{ex3dup}$ isoform did not induce androgen dependent gene

transcription in AR dependent genes such as PSA, TMPRSS2, and maspin. However, the truncated AR isoforms ($AR^{ex1/2/2b}$ or $AR^{ex1/2/3/2b}$) induced constitutive, yet selective ligand independent transcription of hK2, TMPRRSS2, PSA, and NKX3.1. Comparison of $AR^{ex3dup}$ with the truncated AR isoforms ($AR^{ex1/2/2b}$ or $AR^{ex1/2/3/2b}$) showed that the truncated isoforms mediated the androgen independent cell proliferation and growth of 22Rv1 cell but not apoptosis. The truncated AR isoforms showed constitutive activity in the absence of androgens. Further, they showed that the truncated AR isoforms ($AR^{ex1/2/2b}$ or $AR^{ex1/2/3/2b}$) are not specific to 22Rv1, but detectable in other prostate cancer cell lines such as LNCaP, VCaP, and LAPC4. They also tested whether androgen ablation would select for prostate cancer cells that express truncated isoform by xenografting LuCaP23.1 and LuCaP35. They showed that both full length and truncated AR isoforms were elevated in the hormone refractory tumors of both LuCaP23.1 and LuCaP35.

The aforementioned recent reports suggest that the AR NTD plays a role in the hormone refractory stage of prostate cancer. The fact that current androgen deprivation therapy eventually fails makes it urgent to search for an alternative means of therapeutic interventions to prevent or delay the progression to this highly deadly stage of prostate cancer. Therefore, it is of utmost importance to study the mechanisms of AR NTD regulation to discover a novel approach to generate alternative means of therapy. The fact that truncated forms of AR coexist with full length AR under the selective pressure of androgen deprivation therapy implies that AR NTD activation may be a driving force in transition to the hormone refractory stage. In this study, we will try to understand the mechanism of AR NTD activation through the biological and functional characterization of phosphorylation site mutations of truncated AR.

## 1.7 Role of Ack1 in Hormone Refractory Prostate Cancer

The human Ack1 (activated cdc42-associated tyrosine kinase 1) gene is located on chromosome 3q29 which is implicated in adenocortical carcinoma (Dohna et al., 2000) and hematologic neoplasia (Slavutsky et al., 1986). Ack1 mRNA is ubiquitously expressed and its protein expression was detected in brain, thymus, spleen, fat, heart, skin, testis, and liver tissues (Galisteo et al., 2006). Ack1, 120-kDa intracellular non-receptor tyrosine kinase, was initially identified as a binding partner of the activated GTP-bound Cdc42, a small G protein (Manser et al., 1993). Subsequently, Mott et al.(1999) and Cerione et al. (2004) identified the region of Ack1 that interacts with the GTPase Cdc42 as a specific domain of 42 amino acids called Cbd (Cdc42 binding domain) (Cerione, 2004; Mott et al., 1999). Ack1 consists of an N-terminal protein tyrosine kinase (PTK) domain, a Src homology 3 (SH3) domain, a Cdc42/Rac interactive binding (CRIB) domain, and a C-terminal proline-rich region. Amongst the Cdc42 associated proteins, Ack1 specifically interact with activated GTP-bound Cdc42 through its CRIB domain, but not Rac (Kiyono et al., 2000). The other effector proteins such as serine-theroine kinase PAK interacts both with activated GTP-bound Cdc42 and Rac (Morreale et al., 2000).

Several growth factors activated Ack1, including epidermal growth factor receptor (EGFR), M3 muscarinic receptor, integrin, and proteoglycans (Eisenmann et al., 1999a; Linseman et al., 2001; Satoh, 1996; Yang and Cerione, 1997; Yang et al., 1999). In prostate cancer cells, Ack1 activity was enhanced by two receptor tyrosine kinases, Mer and HER2 (Grovdal et al., 2008; Mahajan et al., 2005). However, the exact mechanisms of upstream signaling that activate Ack1 still need to be elucidated.

Ack1 preferentially binds to cdc42 in its GTP bound active form through a unique site 47 amino acid C-terminal to an SH3 domain and inhibits both the intrinsic and GAP-stimulated GTPase activity of cdc42. There are several mechanisms that override the auto inhibitory mode of Ack1 tyrosine kinase such as mutations, amplification, and overexpression (van der Horst et al., 2005). The constitutively active form of Ack1 (caAck1) was made by mutating Leucine-487 to Phenylalanine which abrogates the regulatory effect of the intramolecular interaction between the kinase and the cdc42/Rac interactive binding domains. The constitutively active form of Ack1 (caAck1) significantly enhanced the androgen independent growth of prostate cancer LNCaP and LAPC-4 xenograft tumor growth in mice (Mahajan et al., 2007; Mahajan et al., 2005).

There are several reports that suggested a role for Ack1 in cell spreading (Eisenmann et al., 1999b), vesicle trafficking (Teo et al., 2001), axonal guidance (Worby et al., 2002), dorsal closure in *Drosophila melanogaster* (Bousema et al., 2000), and metastasis (van der Horst et al., 2005). Ack1 was found to be recruited to the EGFR or PDGFR upon EGF or PDGF stimulation (Galisteo et al., 2006) and Grb2 had a role in activation of Ack1 by EGF.

Ack1 has a role in regulation of endocytosis and intracellular sorting of the EGFR. Starkova et al. reported that overexpression of Ack1 enhanced the degradation of the EGFR, and siRNA knock down of Ack1 inhibited EGFR degradation (Starkova et al., 2007). Grovdal et al. showed that overexpression of Ack1 inhibited endosomal sorting of activated EGFR, thereby EGF induced degradation of EGFR was depend upon the activity of Ack1 (Grovdal et al., 2008).

Elevated expression of Ack1 has been detected in several cancers including protate cancer. Van der Horst et al. (2005) reported that Ack1 gene amplification, determined using

comparative genomic hybridization (CGH), led to increased mRNA expression. However the majority of tumors overexpressed Ack1 in the absence of gene amplification (van der Horst et al., 2005). Ovarian and lung tumors exhibit the highest frequency of amplification. Ack1 overexpression is frequently associated with metastatic hormone refractory tumors (10/13 samples showed 5-100-fold overexpression, yet none of the 64 primary prostate tumors had Ack1 gene amplification (van der Horst et al., 2005). The overexpression of Ack1 in breast cancer cell lines led to increased invasiveness and mortality. The data from their *in vivo* studies suggests that Ack1 has a role in extravasation step of metastasis and increased mortality. They suggested that α3β1 integrin ligations activate Ack1 that led to increased Rac activity and cell motility.

There are several recent reports that show the role of Ack1 in different aspects of carcinogenesis and tumor formation such as cell survival. MacKeigan et al. reported that Ack1 function as an anti-apoptotic gene in an RNAi screen targeting against human kinases (MacKeigan et al., 2005). Mahajan et al. showed that Ack1 enhanced growth of prostate cancer xenograft tumors by inducing degradation of tumor suppressor Wwox, hence abrogating Wwox's apoptotic effects (Mahajan et al., 2005). Ack1 is also implicated in survival of v-Ras transformed cells (Nur-E-Kamal et al., 2005).

The aberrant protein tyrosine kinase Ack1 activation has been linked to prostate cancer (Mahajan et al., 2007; van der Horst et al., 2005). Initial proteomic screening identified Ack1 as a binding partner of MER, a receptor tyrosine kinase highly expressed in prostate (Graham et al., 1994; Mahajan and Earp, 2003) . The constitutively active form of Ack1 (caAck), defined by a L487F mutation that relieves autoinhibition of kinase (Kato et al., 2000), dramatically increased prostate xenograft tumor growth in castrated mice (Kato et

al., 2000; Mahajan et al., 2005). Furthermore, a survey of primary tumor samples for activated Ack1 revealed that androgen independent prostate cancer specimens had a higher level of activated Ack1 expression suggesting a role in tumorigenesis. The activated Ack1 induced the AR transcriptional activity of AR regulated genes by enhancing AR recruitment into the promoter of these genes. Their *in vitro* and *in vivo* data showed that activated Ack1 bound and phosphorylated AR at tyrosine residues (i.e. Tyr-267, Tyr-363) (Figure 1.3). Furthermore, they identified a role of Ack1 in heregulin dependent activation of AR. Heregulin treatment of both LNCaP and LAPC4 cells led to phosphorylation of both Ack1 and AR. Further, they showed that AR phosphorylation and activation in response to heregulin treatment was dependent on Ack1 activity. The knockdown of endogenous Ack1 in LNCaP and LAPC4 cells showed that AR recruitment to the enhancer or promoter regions of AR regulated genes (i.e. PSA) was dependent on Ack1. Mass spectrometric analysis of putative Ack1 mediated phosphorylation target tyrosine sites revealed that tyrosine phosphorylation at Try-267 was a major site of AR phosphorylated by Ack1. Further characterization of Ack1 phosphorylation target tyrosine residues showed that mutating tyrosine 267 and 363 to phenylalanine dramatically decreased AR transcriptional activity and recruitment to the enhancer regions of AR target genes and Ack1 driven androgen independent LNCaP cell xenografts tumor growth.

In addition to Ack1, Src kinase phosphorylates AR at Tyr-534 and activates AR under the androgen depleted condition (Guo et al., 2006). The Src mediated AR phosphorylation at Tyr-534 resulted in promoting the growth of prostate cancer cells in the low androgen environment, recruitment of AR to the promoter regions of AR target genes and nuclear localization of AR protein.

## 1.8 Dissertation Research Objectives

Continued AR signaling activity throughout prostate cancer development has become a common theme. The mechanisms of AR activation under HRPC are being actively studied by many research groups. Along with results from *in vitro* and *in vivo* experiments conducted in our laboratory, the elevated levels of phosphorylated Ack1 and AR proteins were prominent feature of HRPC samples compared to benign prostate hyperplasia (BPH) and androgen dependent prostate cancer (Mahajan et al., 2007). The previously identified Ack1 target phosphorylation sites (Tyr-267 and Tyr-363) need to be functionally characterized in order to understand the significance of AR phosphorylation in HRPC. By studying the functional significance of Ack1 target phosphorylation sites of AR, we will be able to contribute to the current state of knowledge about the mechanisms of androgen independent AR phosphorylation and activation under the androgen deprivation condition. The objective of this research were: (1) to understand the role of AR phosphorylation sites (Tyr-267 and Tyr-363) in AR transcriptional activity of full length AR as well as truncated AR missing ligand binding domain (LBD); (2) to determine the role of AR phosphorylation sites on the AR driven cellular phenotypes in cells stably overexpressing AR; (3) to identify the global gene expression changes in LNCaP cells overexpressing either full or truncated mutated AR expression constructs.

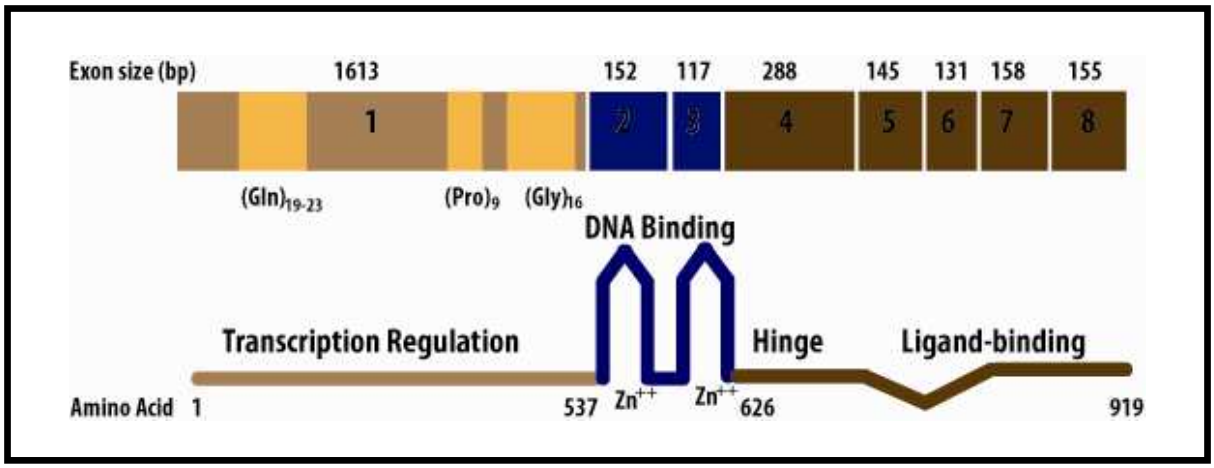


**Figure 1.1. Androgen receptor (AR) modular structure and functional domains.** AR is consisted of 8 exons encoding four functional domains: N-terminal domain, DNA binding domain, hinge region, and ligand binding domain (Adapted from Gelmann, 2002).

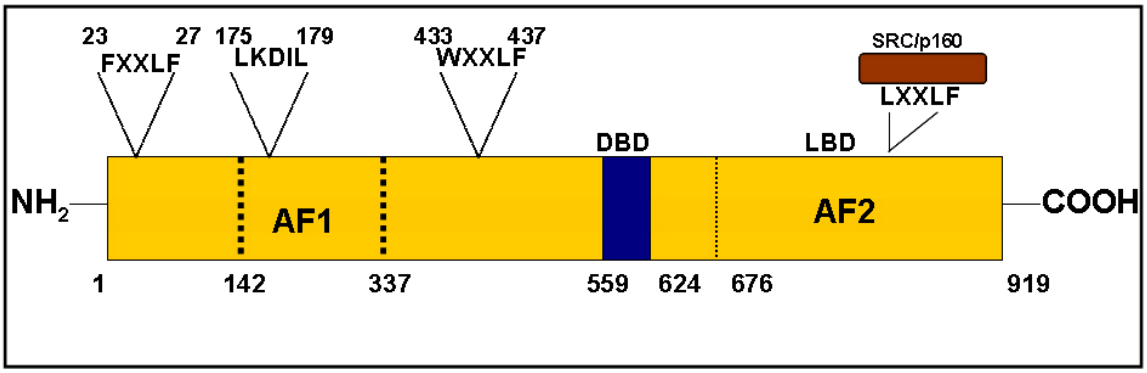


**Figure 1.2. AR modular structure and coactivator interacting motifs.** AR consists of $NH_2$-terminal region (NTD), a DNA binding domain (DBD), a ligand binding domain (LBD). AF1 is a predominant activating function domain located in NTD. Ligand dependent AF-2 interacts with two motifs (FXXLF and WXXLF) motifs in the NTD. LKDIL is a core motif that mediates transcriptional activity of TAU1. The coregulators such as SRC/p160 interacts with AF-2 through the LXXLF motifs (Adapted from Wilson, 2007).

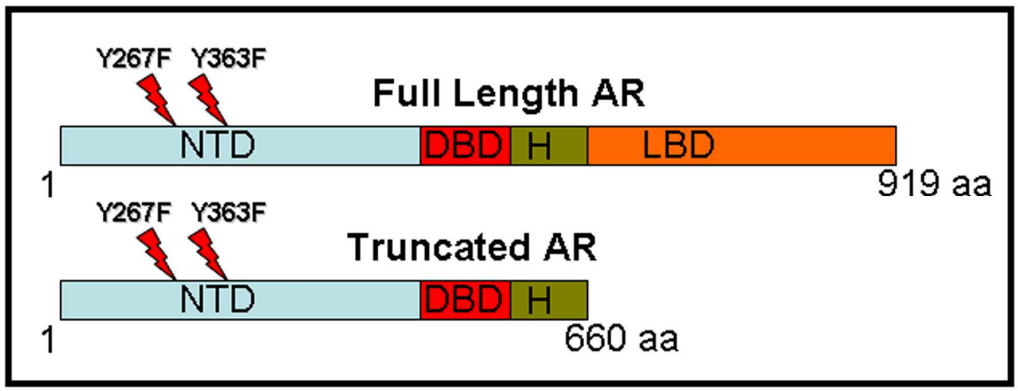


**Figure 1.3. Full length and truncated AR proteins bearing Y267F and Y363F mutations.** Ack1 target phosphorylation sites Y267 and Y363 were mutated into Y267F and Y363F. NTD: N-Terminal domain; DBD: DNA binding domain; H:Hinge region, and LBD: Ligand binding domain.

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# CHAPTER II

## DASATINIB INHIBITS SITE-SPECIFIC TYROSINE PHOSPHORYLATION OF ANDROGEN RECEPTOR BY ACK1 AND SRC KINASES

This chapter has been adapted from: Liu Y, **Karaca M**, Zhang Z, Gioeli D, Earp HS, and Whang YE . Dasatinib inhibits site-specific tyrosine phosphorylation of androgen receptor by Ack1 and Src kinases. Oncogene 2010. (29): 3208-3216.

## 2.1 Abstract

Activation of androgen receptor (AR) may play a role in the development of castration resistant prostate cancer. Two intracellular tyrosine kinases, Ack1 (activated cdc42-associated kinase) and Src, phosphorylate and enhance AR activity and promote prostate xenograft tumor growth in castrated animals. However, the upstream signals that activate these kinases and lead to AR activation are incompletely characterized. In this study, we investigated AR phosphorylation in response to non-androgen ligand stimulation using phospho-specific antibodies. Treatment of LNCaP and LAPC-4 cells with epidermal growth factor (EGF), heregulin, Gas6 (ligand binding to Mer receptor tyrosine kinase and activating Ack1 downstream), interleukin (IL)-6 or bombesin stimulated cell proliferation in the absence of androgen. Treatment of LNCaP and LAPC-4 cells with EGF, heregulin, or Gas6 induced AR phosphorylation at Tyr-267; IL-6 or bombesin treatment did not. AR phosphorylation at Tyr-534 was induced by treatment with EGF, IL-6 or bombesin, but not by heregulin or Gas6. siRNA-mediated knockdown of Ack1 or Src showed that Ack1 mediates heregulin- and Gas6-induced AR Tyr-267 phosphorylation whereas Src mediates Tyr-534 phosphorylation induced by EGF, IL-6, and bombesin. Dasatinib, a Src inhibitor, blocked EGF-induced Tyr-534 phosphorylation. In addition, we show dasatinib also inhibited Ack1 kinase. Dasatinib inhibited heregulin-induced Ack1 kinase activity and AR Tyr-267 phosphorylation. Dasatinib inhibited heregulin-induced AR-dependent reporter activity. Dasatinib also inhibited heregulin-induced expression of endogenous AR target genes. Dasatinib inhibited Ack1-dependent colony formation and prostate xenograft tumor growth in castrated mice. Interestingly, Ack1 or Src knockdown or dasatinib did not inhibit EGF-induced AR Tyr-267 phosphorylation or EGF-stimulated AR activity, suggesting the

existence of an additional tyrosine kinase that phosphorylates AR at Tyr-267. These data suggest that specific tyrosine kinases phosphorylate AR at distinct sites and that dasatinib may exert anti-tumor activity in prostate cancer through inhibition of Ack1.

## 2.2 Introduction

Androgen deprivation therapy through surgical or medical castration is commonly used as a systemic therapy of advanced prostate cancer because it is initially effective in causing tumor regression and palliating cancer-related symptoms. However, virtually all patients eventually progress and develop hormone refractory prostate cancer or castration resistant prostate cancer (CRPC), a terminal disease with poor prognosis. Substantial evidence indicates that androgen receptor (AR) activation in the environment of low circulating testosterone plays a critical role in the development of CRPC. Studies using xenograft tumor models demonstrate that prostate xenograft tumors that recur following castration express AR-dependent genes (Gregory et al., 1998). In these cells, the AR protein is stabilized and constitutively localized to the nucleus and is hypersensitive to low levels of androgen (Gregory et al., 2001). Overexpression of AR enhanced the ability of androgen-dependent prostate xenografts to form tumor in castrated animals while knockdown of AR inhibited their tumorigenicity (Chen et al., 2004). Multiple mechanisms have been shown to activate AR in CRPC. These include AR gene amplification, increased AR expression, AR point mutations frequently occurring in the ligand binding domain that broaden ligand specificity, overexpression of AR coactivators, and intratumoral production of androgen (Scher and Sawyers, 2005).

In addition to these mechanisms, AR may be activated by crosstalk with signaling pathways initiated by cell surface receptors binding to growth factors, cytokines, and neuropeptides. Intracellular pathways involving mitogen activated protein kinase (MAPK) or phosphatidylinositol 3-kinase/Akt have been proposed to mediate activation of AR downstream of receptor tyrosine kinases (Gregory et al., 2004; Wang et al., 2007). For example, epidermal growth factor (EGF) receptor enhances AR transcriptional activity through increasing the interaction between AR and the steroid receptor coactivator TIF2 secondary to MAPK-dependent phosphorylation of TIF2 (Gregory et al., 2004). HER-2 (ErbB2) activates the AR transcriptional function by increasing protein stability, recruitment and DNA binding (Liu et al., 2005; Mellinghoff et al., 2004). AR protein is phosphorylated at multiple serine/threonine residues and phosphorylation at some of these sites has been proposed to regulate nuclear localization and export (Gioeli et al., 2006; Gioeli et al., 2002; Ponguta et al., 2008). Recently, several groups reported that tyrosine phosphorylation of AR protein by nonreceptor tyrosine kinases Src and Ack1 (activated cdc42-associated kinase) may play a role in AR activation in the low androgen environment, thereby promoting the development of CRPC (Guo et al., 2006; Kraus et al., 2006; Mahajan et al., 2007). Src-mediated phosphorylation of AR at Tyr-534 resulted in activation of AR and nuclear translocation and DNA binding in the absence of androgen (Guo et al., 2006; Kraus et al., 2006). Our previous work identified Ack1 as a protein activated downstream of Mer receptor tyrosine kinase in prostate cancer cells (Mahajan et al., 2005). Furthermore, we demonstrated that expression of activated Ack1 increased prostate xenograft tumor growth in castrated animals as well as AR target gene expression and AR recruitment to the enhancers of target genes at suboptimal androgen concentrations (Mahajan et al., 2007). Ack1

interacted with and phosphorylated AR protein at Tyr-267 and Ack1 was shown to be required for optimal AR target gene expression and AR recruitment. Tyrosine phosphorylated AR protein was expressed in 8 out of 18 primary CRPC tumor samples and its expression was correlated with Ack1 activation in the same tumors. In this report, we investigate the upstream signals activating AR phosphorylation and we show that Src and Ack1 kinases each contribute to site-specific phosphorylation of AR.

## 2.3 Materials and Methods

**Cells and Reagents.** LAPC-4 cells were provided by Dr. Charles Sawyers (Klein et al., 1997). EGF (R&D Systems, Minneapolis, MN), IL-6 (R&D), Gas6 (R&D), and bombesin (Sigma-Aldrich, St. Louis, MO) were purchased. Heregulin was a gift from Genentech (South San Francisco, CA). Dasatinib was obtained from Bristol-Myers-Squibb (Princeton, NJ). Phospho-specific polyclonal antibody against Tyr-267 of AR was generated by a commercial vendor (21st Century Biochemicals, Marlboro, MA). Rabbits were immunized with carrier-conjugated phospho-peptides spanning Tyr-267. Immunodepletion using a nonphospho-peptide column and affinity purification using the phospho-peptide column were performed by the vendor. Phospho-specific antibody against Tyr-534 of AR was raised in rabbits using standard methods and affinity purified in a similar fashion; its characterization has been reported (DaSilva et al., 2009). A mouse monoclonal antibody against total AR (F39.4.1, Biogenex, San Ramon, CA) was used for immunoblotting. A polyclonal antibody against AR (C-19, Santa Cruz) was used for immunoprecipitation. The antibody against total Ack1 was described previously (Mahajan et al., 2005). A phospho-specific antibody against Ack1 p-Tyr-284 (# 09-142) was obtained from Millipore (Billerica,

MA). Antibodies against total Src (#2108) and phospho-specific Src p-Tyr-416 (#2101) were obtained from Cell Signaling Technology (Beverly, MA).

**Transfection and Knock Down.** 293T cells and COS7 cells were transfected with AR or Ack1 or Src expression vectors using Effectene (Qiagen, Valencia, CA) according to the manufacturer's direction. siRNA sequences against Ack1 were previously described (Mahajan et al., 2007). For knocking down Src, Validated Stealth RNAi™ siRNA against Src (Invitrogen, Carlsbad, CA) was used according to the manufacturer. LNCaP cells were transfected using siPort Lipid (Ambion, Austin, TX) with 100 nM of siRNA or negative control scrambled siRNA. After 24 hrs, cells were treated with ligands as indicated. All experiments were repeated at least three times.

**Proliferation Assays.** LNCaP cells or LAPC-4 cells were seeded at a density of $10^4$ cells per well in triplicate in a 96-well plate in serum-free medium (RPMI 1640 for LNCaP and IMDM for LAPC-4). On day 0, cells were treated with DHT (10 nM), EGF (100 ng/ml), heregulin (10 ng/ml), Gas6 (100 ng/ml), bombesin (1 nM), or IL-6 (10 ng/ml). After 3 days of incubation, relative cell proliferation was measured using colorimetric dye WST-8 (Cell Counting Kit-8, Dojindo, Rockville, MD) according to the manufacturer's direction.

**Western Blot Analysis.** Whole-cell lysates were prepared on ice using Mammalian Protein Extraction Reagent (M-PER) (Pierce Biotechnology, Rockford, IL) according to manufacturer's instructions. Cytoplasmic extracts were prepared as previously described (Mayo et al., 1997). Protein extracts were quantified by Bradford assay (Bio-Rad Laboratories, Hercules, CA) and analyzed by SDS-PAGE as previously described (Steinbrecher et al., 2005).

**Reporter Assays and Quantitative RT-PCR.** LNCaP cells (8 X $10^5$ cells per 6-cm plate) were transfected with the ARR2-PB-luciferase reporter (500 ng) (Zhang et al., 2000) along with the AR expression vector (50 ng), using Effectene, as described (Mahajan et al., 2007). After overnight incubation, cells were pre-treated with dasatinib as indicated for 2 hrs, then EGF (100 ng/ml) or heregulin (10 ng/ml) for 20 hrs. Luciferase activity was determined, as described (Mahajan et al., 2007). The IC50 concentration of dasatinib required for inhibition of heregulin-driven luciferase activity was calculated using the dose-response variable slope (four parameters) function of the GraphPad Prism 5 software (GraphPad, La Jolla, CA). For measurement of PSA and hK2 mRNA levels, LNCaP or LAPC-4 cells were incubated in serum-free medium and were pre-treated with dasatinib (10 nM) for 2 hrs, then treated with heregulin (10 ng/ml) or EGF (100 ng/ml) for 20 hrs. Total RNA was isolated and the mRNA levels of PSA and hK2 were determined by quantitative RT-PCR, as described (Mahajan et al., 2007).

**Colony Formation in Soft Agar.** LNCaP cells expressing Ack1 or vector control ($10^4$ cells per well) were suspended in 0.45% Noble agar prepared in growth medium along with the indicated concentration of dasatinib and placed above a layer of solidified 0.9% Noble agar in 6 well plates in triplicates. After 3 weeks, colonies were visualized by staining with MTT.

**Xenograft Tumor Growth**. LNCaP cells stably expressing activated Ack1 by retroviral transduction have been described (Mahajan et al., 2005). LNCaP-Ack1 cells (2 X $10^6$) were mixed with an equal volume of Matrigel (BD Biosciences, Franklin Lakes, NJ) and implanted subcutaneously in the flank of castrated nude male mice, as described (Mahajan et al., 2007; Mahajan et al., 2005). When tumors became palpable, mice were randomly

divided into two groups. Dasatinib was dissolved in citrate buffer (80 mM, pH 3.1) and administered to mice via oral gavage at a dose of 30 mg/kg twice daily. Tumor size was measured with calipers twice per week. These procedures were approved by the Institutional Animal Use and Care Committee.

**Statistical Methods.** The nonparametric Wilcoxon rank-sum test (using Van der Waerden normal scores) was used for the two-group comparisons of tumor volumes of the treated group (n=8) to the control group (n=7) at days 25 and 29 of treatment. Exact nominal (unadjusted for multiple comparisons) two-sided p-values were reported. Statistical analyses were performed using SAS statistical software, Version 9.2 (SAS Institute, Inc., Cary, NC).

## 2.4 Results

**Site-specific Phosphorylation of Androgen Receptor by Ack1 and Src Kinases.** Previous reports demonstrated that AR protein may be phosphorylated at Tyr-534 or Tyr-267 residues (Guo et al., 2006; Kraus et al., 2006; Mahajan et al., 2007). To elucidate signaling pathways leading to AR tyrosine phosphorylation, phospho-specific antibodies against AR protein phosphorylated at these residues were generated. Their specificity was tested against wildtype and phosphorylation-site mutant AR. Wildtype AR, Y267F AR, or Y534F AR expression vectors were co-transfected along with the activated Ack1 or activated Src kinase expression vector. The phospho-Y267 specific antibody detected AR only when Ack1 was co-expressed and did not detect the AR Y267F mutant protein in cell lysates co-expressing Ack1 (Figure 2.1A). The phospho-Y534 specific antibody detected AR only when Src was co-expressed and did not recognize the AR Y534F mutant protein in the presence of Src (Figure 2.1B). Co-expression of Ack1 led to phosphorylation of Tyr-267 but not Tyr-534 of AR. Conversely, co-expression of Src resulted in phosphorylation of Tyr-534 but not Tyr-

267 of AR. These data suggest that Ack1- and Src-mediated phosphorylation of AR is site-specific (i.e. Ack1 targets Tyr-267 but not Tyr-534 and vice versa for Src).

**Phosphorylation of Androgen Receptor by Ligand Stimulation.** Tyrosine phosphorylation of endogenous AR in prostate cancer cells induced by physiologic ligand stimulation was characterized using the phospho-specific antibodies. Extracellular ligands that bind to the cell surface receptors and have previously been reported to enhance AR activity or may potentially enhance AR activity were tested for their ability to induce AR tyrosine phosphorylation. Gas6 is the ligand for Mer receptor tyrosine kinase, which we have previously shown to activate Ack1 in prostate cells (Mahajan et al., 2005). Heregulin binds to HER-3 and activates HER-2 kinase through the formation of the HER-2/HER-3 heterodimer. Bombesin (also known as gastrin-releasing peptide) is a neuropeptide produced by neuroendocrine cells, which has been reported to support growth of androgen-dependent LNCaP cells in an androgen-depleted condition through Src-mediated activation of AR (Desai et al., 2006; Gong et al., 2006; Yang et al., 2009). Interleukin-6 leads to ligand-independent activation of the AR N-terminal domain that involves MAPK and steroid receptor coactivator-1 (Ueda et al., 2002b). Treatment of LNCaP cells with EGF, heregulin, or Gas6 induced phosphorylation of the AR protein at Tyr-267 (Figure 2.1C). AR phosphorylation was transient and peaked at 60-90 min. and returned to the basal level by 4 hrs (data not shown). Treatment with IL-6 or bombesin did not induce phosphorylation of the AR at Tyr-267. Phosphorylation of the AR at Tyr-534 was induced by treatment with EGF, IL-6 or bombesin, but not with heregulin or Gas6 (Figure 2.1D). EGF was the only ligand tested that induced AR phosphorylation at both Tyr-267 and Tyr-534 sites, in contrast to other ligands that produced site-specific AR phosphorylation (i.e. heregulin and Gas6 at

Tyr-267 and IL-6 and bombesin at Tyr-534). LAPC-4 prostate cancer cells that express wildtype AR endogenously (Klein et al., 1997) were treated with these ligands and AR phosphorylation at Tyr-267 and Tyr-534 sites was analyzed similarly. LAPC-4 cells exhibited the same pattern of AR phosphorylation by these ligands as LNCaP cells (Figure 2.2).

**Stimulation of Cell Proliferation by Non-androgen Ligands.** Since cell proliferation requires AR activation in androgen-dependent prostate cancer cells, the effect of these ligands inducing AR phosphorylation on cell proliferation was determined. In LNCaP cells, treatment with EGF, heregulin, Gas6, bombesin, or IL-6 all stimulated cell proliferation to similar levels as the androgen dihydrotestosterone (DHT) (Figure 2.3). In LAPC-4 cells, all ligands stimulated cell proliferation although Gas6 and IL-6 were less effective than DHT or other ligands. These results are in agreement with previous reports of stimulation of proliferation in the absence of androgen by heregulin (Gregory et al., 2005), bombesin (Desai et al., 2006; Lee et al., 2001), or IL-6 (Ueda et al., 2002a). These data demonstrate that these non-androgen ligands inducing AR phosphorylation promote prostate cancer cell proliferation in the absence of androgen.

**Dasatinib Inhibits Ack1 Kinase Activity but does not Inhibit EGF-induced AR Phosphorylation at Tyr-267.** For characterization of signaling pathways and therapeutic applications, a small molecule kinase inhibitor of Ack1 would be desirable. A previous report indicated that dasatinib, a potent inhibitor of Src and Abl kinases, interacted with several additional tyrosine kinases in a competition binding assay, including Ack1 (Carter et al., 2005). The ability of dasatinib to inhibit Ack1 autophosphorylation as a marker of kinase activity was tested in 293T cells transfected to express activated Ack1 (Figure 2.4A).

Dasatinib inhibited Ack1 autophosphorylation in a dose dependent manner, with dasatinib doses of 10 nM or greater demonstrating nearly complete inhibition of Ack1 phosphorylation, consistent with the previously reported Kd of 6 nM (Carter et al., 2005). Treatment of LNCaP cells with dasatinib inhibited heregulin-induced Ack1 phosphorylation almost completely at doses of 10 nM or greater (Figure 2.4B). Dasatinib treatment inhibited heregulin-induced AR phosphorylation at Tyr-267. The concentration of dasatinib required for inhibition of AR phosphorylation at Tyr-267 was similar to that for inhibition of heregulin-induced Ack1. This result is consistent with the notion that heregulin-induced activation of Ack1 mediates AR phosphorylation at Tyr-267. The effect of dasatinib on EGF-induced phosphorylation of AR was tested. EGF-induced AR phosphorylation at Tyr-534 was inhibited by dasatinib (Figure 2.5A). Since dasatinib inhibits Src, this finding is consistent with the idea that Src activation downstream of EGF receptor leads to AR phosphorylation at Tyr-534. Dasatinib had no effect on EGF-induced AR phosphorylation at Tyr-267, even at doses as high as 1000 nM (Figure 2.5B). However, Src activation after EGF treatment was inhibited by dasatinib at a dose as low as 1 nM (Figure 2.5D). In LNCaP cells, Ack1 was not activated after EGF treatment while heregulin-induced Ack1 activation was detected (Figure 2.5C). These results suggest that at least one other tyrosine kinase (other than Src and Ack1) mediates AR phosphorylation at Tyr-267 after EGF treatment in LNCaP cells and that this kinase (or kinases) is insensitive to dasatinib.

**Role of Ack1 and Src in Ligand-stimulated AR Phosphorylation.** To investigate more directly the role of Ack1 or Src in AR phosphorylation after ligand stimulation, the effect of Ack1 or Src knockdown by siRNA was tested (Figure 2.6). Ack1 knockdown inhibited AR phosphorylation at Tyr-267 induced by heregulin or Gas6 treatment. Src

knockdown inhibited AR phosphorylation at Tyr-534 induced by EGF or IL-6 or bombesin treatment. However, EGF-induced AR phosphorylation at Tyr-267 was not inhibited by Ack1 knockdown or Src knockdown, despite markedly reduced expression levels of Ack1 or Src by siRNA confirmed by immunoblotting. These data suggest that downstream of cell surface receptors, Ack1 mediates AR tyrosine phosphorylation at Tyr-267 and Src mediates AR tyrosine phosphorylation at Tyr-534. However, EGF-induced AR phosphorylation at Tyr-267 likely involves an additional kinase(s) that is not inhibited by dasatinib.

**Effect of Dasatinib on Ack1-Induced AR Activation.** The biological effect of dasatinib on prostate cancer cells was investigated. First, LNCaP cells transfected with the AR-dependent reporter ARR2-PB-luciferase were investigated. Treatment of these cells with heregulin or EGF (without androgen) increased luciferase activity. AR antagonist bicalutamide inhibited androgen-induced AR reporter activity but did not inhibit heregulin- or EGF-induced AR reporter activity (Figure 2.7). Therefore, heregulin- or EGF-induced AR activation likely reflects ligand-independent activation of AR via receptor tyrosine kinase-mediated pathways. Dasatinib treatment inhibited heregulin-induced AR reporter activity with the IC50 of 4.9 nM, consistent with the hypothesis that the stimulatory effect of heregulin is mediated by downstream Ack1 activation (Figure 2.8A). However, dasatinib treatment had no effect on EGF-induced AR reporter activity, even at doses as high as 1000 nM, in contrast to its inhibitory effect on heregulin-mediated AR reporter activity (Figure 2.8B). The effect of dasatinib on expression of endogenous AR target genes prostate specific antigen (PSA) and human kallikrein 2 (hK2) (also known as KLK2) was tested. Treatment of LNCaP cells with EGF or heregulin increased PSA and hK2 mRNA levels by 4-6 fold. Dasatinib treatment partially blocked heregulin-induced expression of PSA and hK2 mRNA

but not EGF-induced expression of PSA and hK2 mRNA (Figure 2.8 C and D). Similar results were seen in LAPC-4 cells (Figure 2.9). Since heregulin-induced Tyr-267 phosphorylation is blocked by dasatinib, but EGF-induced Tyr-267 phosphorylation is not blocked by dasatinib, these results suggest that the stimulatory effect of growth factors on AR is correlated with induction of Tyr-267 phosphorylation, either by Ack1 or an unidentified tyrosine kinase downstream of EGF.

**Effect of Dasatinib on Ack1-induced Colony Formation and Xenograft Tumor Growth.** Activated Ack1 enhances the ability of LNCaP cells to form colonies in soft agar and grow as subcutaneous xenograft tumor in castrated mice (Mahajan et al., 2007; Mahajan et al., 2005). The effect of dasatinib on Ack1-induced colony formation was determined. Dasatinib treatment decreased the number of soft agar colonies in LNCaP cells expressing activated Ack1 in a dose-dependent manner (Figure 2.10A). Dasatinib also inhibited soft agar colony growth of LNCaP vector control cells, likely through inhibition of Src kinase expressed in LNCaP (Park et al., 2008). We investigated the effect of dasatinib treatment in vivo on the xenograft tumor growth of LNCaP-Ack1 cells in castrated mice. Castrated mice with xenograft tumors were treated with dasatinib orally or vehicle control and the tumor volume was monitored. Administration of dasatinib resulted in significant inhibition of xenograft tumor growth. At day 25 and 29, the tumor volume of the dasatinib treatment group was decreased by approximately 50% compared to the tumor volume of the control group (Figure 2.10B). The phosphorylation status of Ack1 and AR proteins was determined by immunoblotting of protein extracts of harvested tumor tissues at the conclusion of the experiment. In tumors harvested from control animals, Ack1 and AR at Tyr-267 were constitutively phosphorylated, whereas in tumors from dasatinib treated animals, Ack1 and

AR proteins were not phosphorylated (Figure 2.10C). These data suggest that inhibition of Ack1 kinase by dasatinib leads to loss of AR phosphorylation and decreased xenograft tumor growth.

## 2.5 Discussion

In this report, we demonstrate that Ack1 and Src tyrosine kinases target distinct AR phosphorylation sites (i.e. Tyr-267 by Ack1 and Tyr-534 by Src) after activation by cell surface receptors. EGF, IL-6, and bombesin-induced AR phosphorylation at Tyr-534 was inhibited by Src knockdown and dasatinib, a potent Src inhibitor, suggesting Src involvement downstream of these ligands. Activation of AR by EGF and bombesin through Src had been reported previously (Desai et al., 2006; Guo et al., 2006; Kraus et al., 2006). A recent report indicated that the neuroendocrine-derived peptide parathyroid hormone-related protein stabilizes AR by reducing interaction with the ubiquitin ligase CHIP through Src-mediated phosphorylation of AR at Tyr-534 (DaSilva et al., 2009). Involvement of Ack1 downstream of heregulin and Gas6 in AR phosphorylation at Tyr-267 was confirmed by Ack1 knockdown as well as dasatinib, which has been shown to be an inhibitor of Ack1 in this work. However, inhibition of Src and Ack1 did not prevent EGF-induced AR tyrosine phosphorylation at Tyr-267, suggesting the existence of an additional unidentified tyrosine kinase (or kinases) capable of phosphorylating AR at Tyr-267 (Figure 2.11). Although identification and understanding the functional role of this kinase on AR signaling will require further work, it is clear that Tyr-267 phosphorylation is involved in some modes of AR transactivation no matter which tyrosine kinase is involved. Inhibition of growth factor-induced AR activity by dasatinib correlates with loss of AR phosphorylation at Tyr-267, as dasatinib inhibits heregulin-induced AR Tyr-267 phosphorylation and AR reporter activity

and target gene expression. In contrast, EGF-induced AR Tyr-267 phosphorylation and AR reporter activity and target gene expression are resistant to dasatinib. This result raises a possibility that phosphorylation of Tyr-267 is required for AR activation downstream of receptor tyrosine kinases.

The functional consequences of AR phosphorylation at Tyr-267 and Tyr-534 appear similar in that phosphorylation at both sites has been linked to enhanced AR recruitment and expression of AR target genes at suboptimal androgen concentrations (Guo et al., 2006; Mahajan et al., 2007). However, chromatin immunoprecipitation analysis demonstrated that Src-induced AR activation involves AR recruitment preferentially to the proximal promoter elements of the canonical target gene prostate specific antigen and that there is no increase in the binding of AR to the distal enhancer where androgen-induced and Ack1-driven AR recruitment takes place (Desai et al., 2006; Mahajan et al., 2007; Yang et al., 2009). Whether this difference in the AR recruitment sites stems from the distinct AR phosphorylation sites or possibly due to other components of the transcriptional complex regulated by these kinases is unclear. The differential effect of AR phosphorylation sites on target gene expression and binding of coactivators and corepressors and other proteins involved in the assembly of the active transcriptional complex requires further elucidation.

Dasatinib inhibits a wide spectrum of kinases such as Abl and Src and its ability to inhibit Bcr-Abl kinase (unmutated and mutated forms) led its approval for treatment of imatinib-resistant chronic myelogenous leukemia (Brave et al., 2008). In addition to the ability of Src to promotes castration resistant progression and AR activation, Src is involved in regulating prostate cancer cell migration, invasion, and metastasis and affects bone remodeling (Araujo and Logothetis, 2009; Park et al., 2008). Therefore, dasatinib is

currently being studied in treatment of CRPC as a single agent and in combination with docetaxel chemotherapy (Araujo et al., 2009; Yu et al., 2009). Our data show that Ack1 kinase is inhibited by dasatinib at clinically relevant concentrations, and preclinical xenograft studies demonstrate the feasibility of inhibiting Ack1 *in vivo* as tumors exhibit loss of constitutive Ack1 and AR phosphorylation after oral treatment with dasatinib. This raises a possibility that dasatinib may have clinical activity against Ack1-driven malignancies. Ack1 binds and is activated by several receptor tyrosine kinases such as EGFR, HER-2, Mer, Axl, platelet derived growth factor receptor, LTK (leucocyte receptor tyrosine kinase belonging to the insulin receptor family) and ALK (anaplastic lymphoma kinase) (Galisteo et al., 2006; Mahajan et al., 2005; Pao-Chun et al., 2009). A recent study demonstrated that the Ack1 gene is amplified and overexpressed in several tumor types, including castration resistant prostate cancer, and this was correlated with cancer progression and poor prognosis (van der Horst et al., 2005). Additionally, Ack1 may also be activated by oncogenic mutations. The current release (version 42) of the Catalogue of Somatic Mutation in Cancer database reported 5 out of 229 tumor samples containing point mutations in Ack1, some of which are likely to lead to constitutive activation of kinase (Forbes et al., 2006). In a subset of primary CRPC tumor specimens (8 out of 18), expression of tyrosine-phosphorylated AR and Ack1 was detected by immunoprecipitation and immunoblotting of tumor lysates (Mahajan et al., 2007). Our findings provide additional mechanisms by which dasatinib may exert anti-tumor activity in CRPC. Although both Src and Ack1 phosphorylate AR protein, they target distinct sites. Therefore, phospho-Tyr-267 and phospho-Tyr-534 AR expression in CRPC tumors may serve as a predictive biomarker of tyrosine kinase inhibitor therapy.

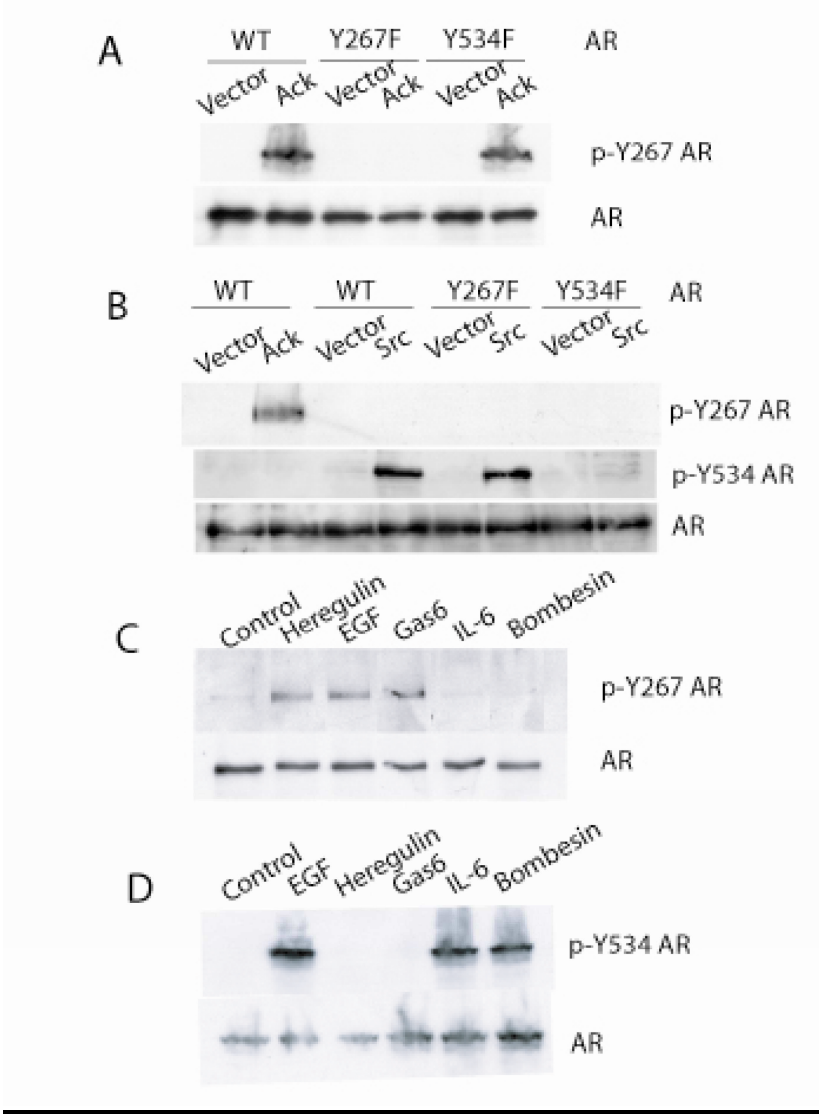


**Figure 2.1. Phospho-specific antibodies demonstrate that AR is phosphorylated at Tyr-267 and Tyr-534 after ligand stimulation.** (A) 293T cells were transfected with the expression vector encoding wildtype AR or Y267F AR or Y534F AR along with constitutively active Ack1 or empty vector. After 24 hours, protein extracts were immunoblotted with the antibody specific for phospho-Y267 AR or total AR. (B) COS7 cells were transfected with the vector encoding wildtype AR or Y267F AR or Y534F AR along with constitutively active Ack1 or activated Src or empty vector, as indicated. After 24 hours, protein extracts were immunoprecipitated with the AR antibody, then immunoblotted with the antibody specific for phospho-Y267 AR or phospho-Y534 AR or total AR, as indicated. (C) LNCaP cells were treated with EGF (100 ng/ml), heregulin (10 ng/ml), Gas6 (100 ng/ml), IL-6 (10 ng/ml), or bombesin (1 nM) for 60 min. Protein extracts were immunoblotted with the antibody specific for phospho-Y267 AR or total AR. (D) LNCaP cells were treated as above. Protein extracts were immunoprecipitated with the AR antibody, then immunoblotted with the antibody specific for phospho-Y534 AR. Blots shown are representative of three independent experiments.

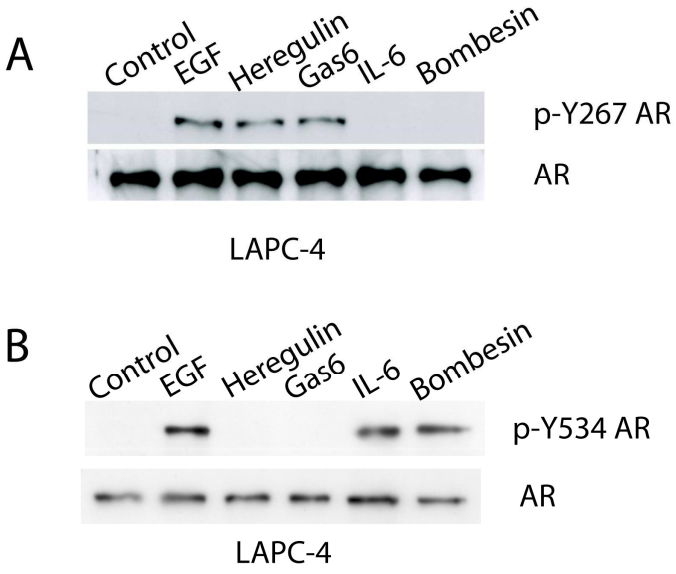


**Figure 2.2. AR is phosphorylated at Tyr-267 or Tyr-534 after ligand stimulation in LAPC-4 cells.** (A, B) LAPC-4 cells were treated with EGF (100 ng/ml), heregulin (10 ng/ml), Gas6 (100 ng/ml), IL-6 (10 ng/ml), or bombesin (1 nM) for 60 min. Protein extracts were immunoblotted with the antibody specific for phospho-Y267 AR or phospho-Y534 AR or total AR, as indicated.

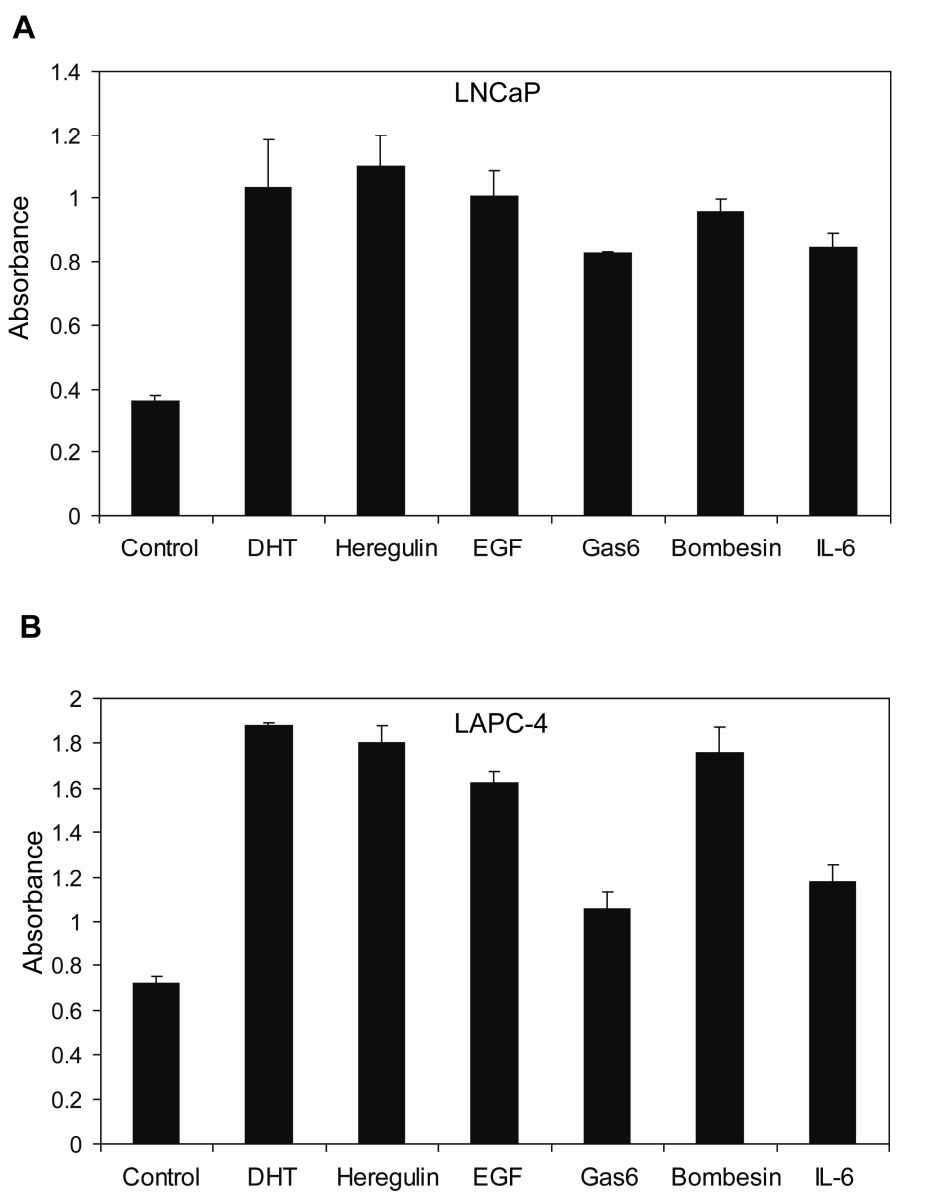


**Figure 2.3. EGF, heregulin, Gas6, bombesin, and IL-6 stimulate proliferation of androgen-dependent prostate cancer cell lines in the absence of androgen.** Cells were seeded in serum-free medium in triplicate wells of a 96-well plate and treated with EGF (100 ng/ml), heregulin (10 ng/ml), Gas6 (100 ng/ml), IL-6 (10 ng/ml), or bombesin (1 nM) for 72 hours. Relative cell proliferation was determined by addition of colorimetric dye WST-8 and measuring absorbance at 450 nM. (A) LNCaP cells. (B) LAPC-4 cells. Data shown are representative of three similar independent experiments. All treatment conditions are significantly different from untreated control with $p < 0.05$ by t-test.

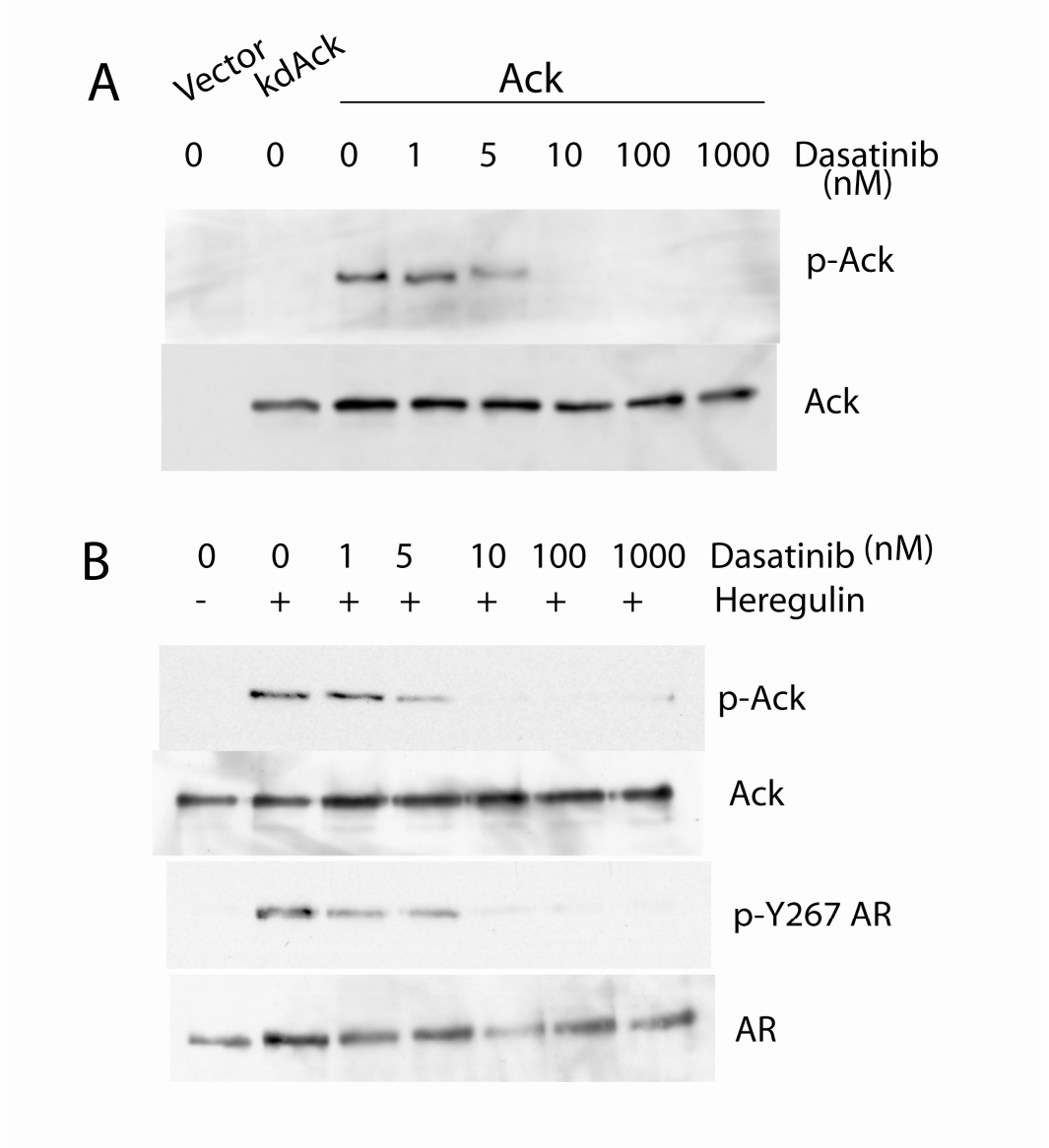


**Figure 2.4. Dasatinib inhibits Ack1 kinase activity and inhibits heregulin-induced AR phosphorylation at Tyr-267.** (A) 293T cells were transfected with empty vector or kinase-dead Ack1 or constitutively active Ack1. After 24 hours, cells were treated with increasing concentrations of dasatinib for 2 hours. Protein extracts were immunoblotted with the antibody against phospho-Ack1 or total Ack1. (B) LNCaP cells were pre-treated with dasatinib at increasing concentrations for 2 hours, then treated with heregulin (10 ng/ml) for 60 min. Protein extracts were immunoblotted with the antibody against phospho-Y267 AR or total AR or phospho-Ack1 or total Ack1. Blots shown are representative of three independent experiments.

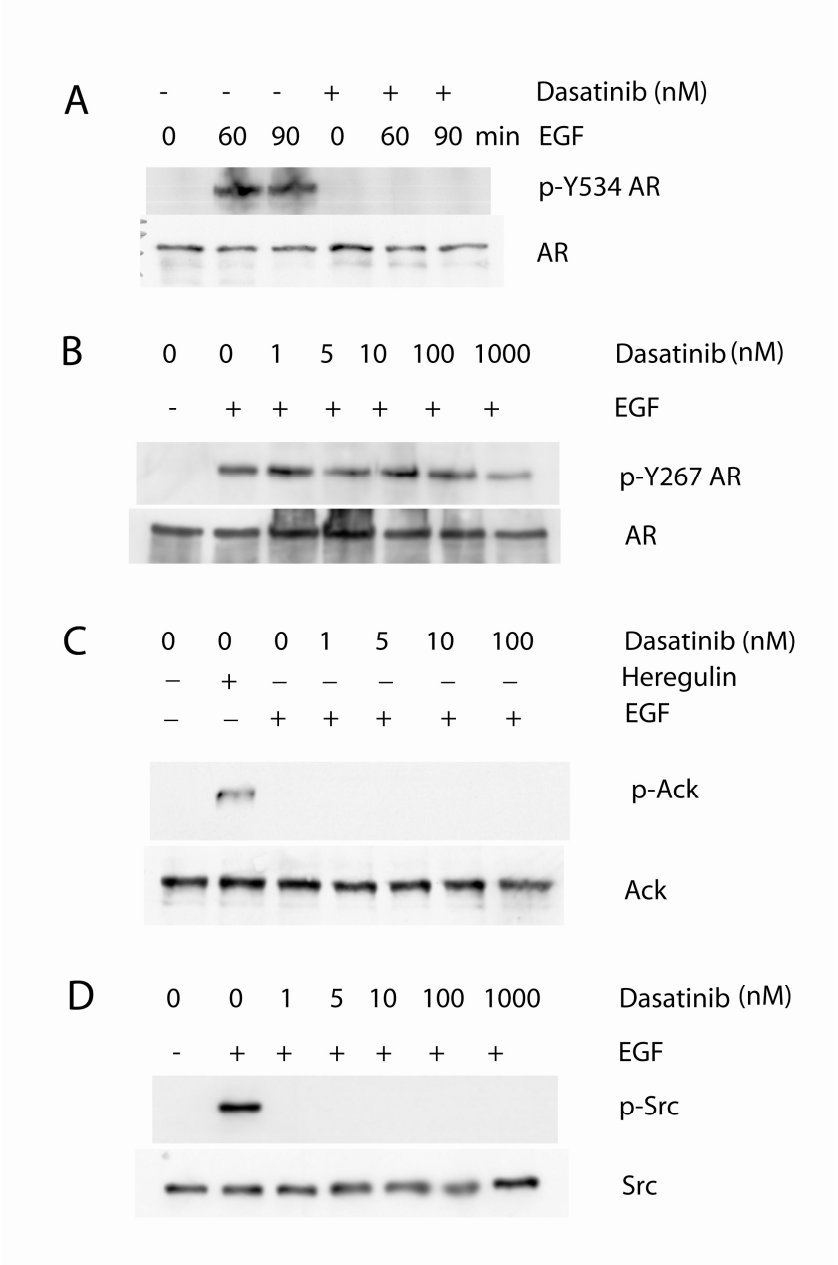


**Figure 2.5. Dasatinib inhibits EGF-induced AR phosphorylation at Tyr-534 but not Tyr-267.** (A) LNCaP cells were pre-treated with dasatinib (10 nM) for 2 hours, then treated with EGF (100 ng/ml) for 60 or 90 min. Protein extracts were immunoprecipitated with the AR antibody, then immunoblotted with the antibody specific for phospho-Y534 AR or total AR. (B) LNCaP cells were pre-treated with dasatinib at increasing concentrations for 2 hours, then treated with EGF (100 ng/ml) for 60 min. Protein extracts were immunoblotted with the antibody against phospho-Y267 AR or total AR. (C, D) LNCaP cells were treated as above. Cells were also treated with heregulin (10 ng/ml) for 60 min as a positive control of Ack1 activation. Protein extracts were immunoblotted with the antibody against phospho-Ack1 or total Ack1 or phospho-Src or total Src. Blots shown are representative of three independent experiments.

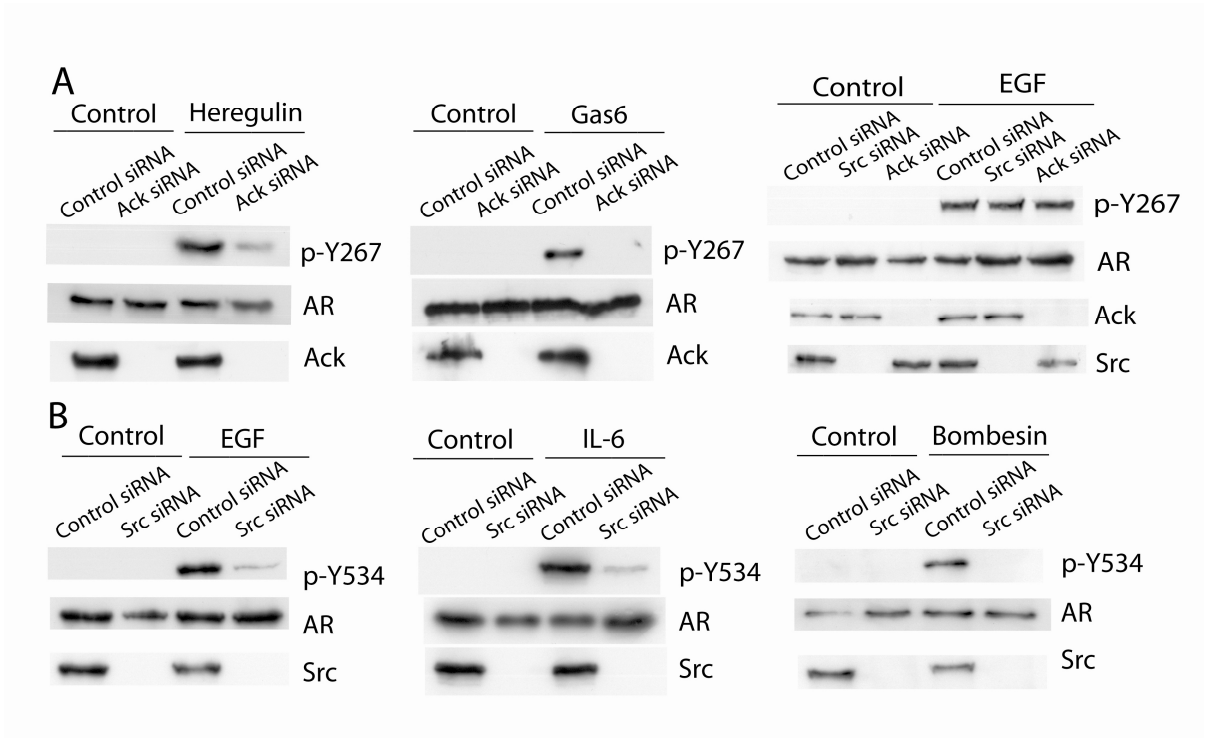


**Figure 2.6. Knockdown of Src or Ack1 demonstrates the existence of an additional kinase that induces AR phosphorylation at Tyr-267 after EGF stimulation.** LNCaP cells were transfected with control or Src-specific or Ack1-specific siRNA. After 24 hours, cells were treated with heregulin, Gas6, EGF, IL-6, or bombesin, as indicated, for 60 min. (A) Protein extracts were immunoblotted with the antibody specific for phospho-Y267 AR or total AR. (B) Protein extracts were immunoprecipitated with the AR antibody, then immunoblotted with the antibody specific for phospho-Y534 AR. Protein extracts were also immunoblotted with the antibody specific for Src or Ack1. Blots shown are representative of at least three independent experiments.

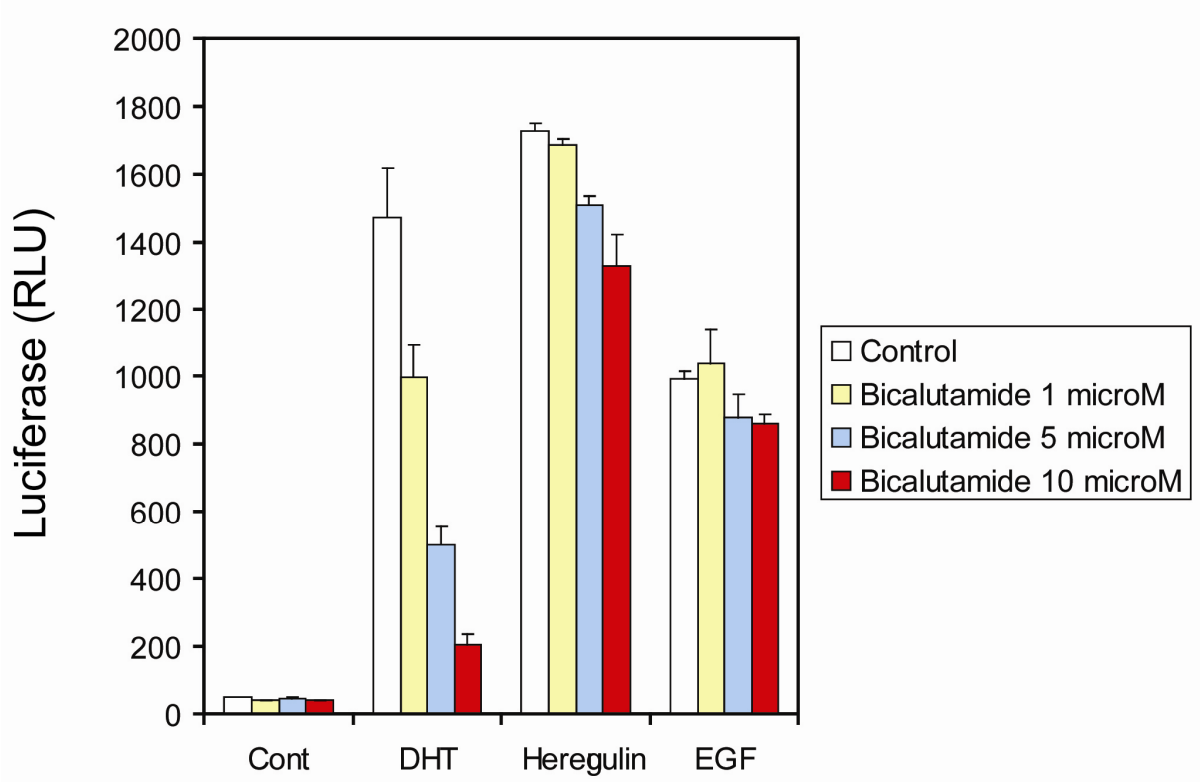


**Figure 2.7. AR antagonist bicalutamide inhibits androgen-induced AR reporter activity but not growth factor-induced AR reporter activity.** LNCaP cells were transfected with the AR reporter ARR2-PB-luciferase. After overnight incubation, cells were pre-treated with bicalutamide for 2 hrs, then with DHT (10 nM), heregulin (10 ng/ml) or EGF (100 ng/ml) for 20 hrs. Cell extracts were assayed for luciferase activity. The mean and standard deviation of triplicate samples of a representative experiment are shown from three independent experiments that showed similar results.

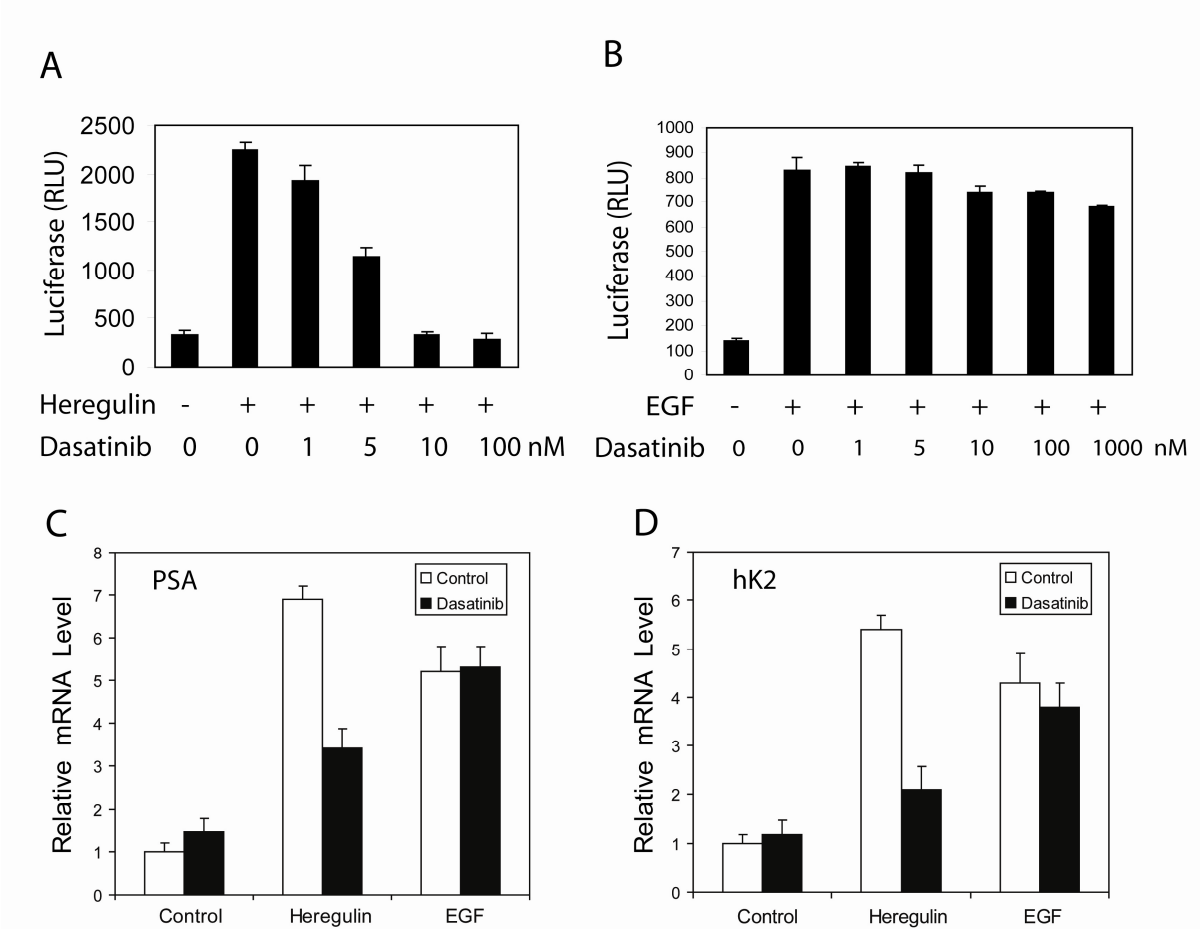


**Figure 2.8. Dasatinib inhibits heregulin-induced AR activity, but not EGF-induced AR activity.** (A, B) LNCaP cells were transfected with the AR reporter ARR2-PB-luciferase. After overnight incubation, cells were pre-treated with dasatinib for 2 hrs, then with heregulin (10 ng/ml) (A) or EGF (100 ng/ml) (B) for 20 hrs. Cell extracts were assayed for luciferase activity. The mean and standard deviation of triplicate samples of a representative experiment are shown from three independent experiments with similar results. (C, D) LNCaP cells were pre-treated with dasatinib (10 nM) for 2 hrs, then with heregulin (10 ng/ml) or EGF (100 ng/ml) for 20 hrs. RNA was isolated and quantitative RT-PCR was performed to determine the levels of PSA (C) or hK2 (D) mRNA. Data shown are representative of two independent experiments with similar results.

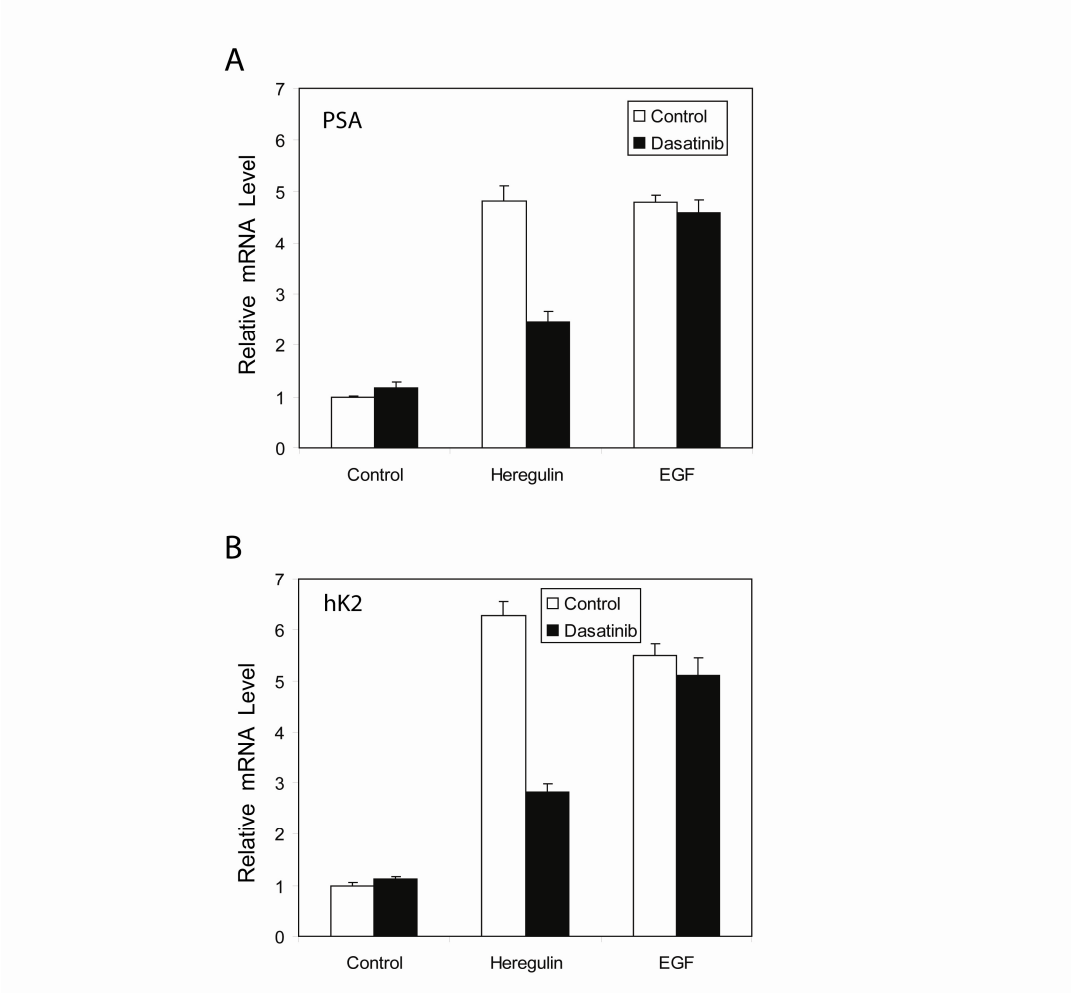


**Figure 2.9. Dasatinib inhibits heregulin-induced expression but not EGF-induced expression of PSA and hK2 mRNA in LAPC-4 cells.** LAPC-4 cells were pre-treated with dasatinib (10 nM) for 2 hrs, then with heregulin (10 ng/ml) or EGF (100 ng/ml) for 20 hrs. RNA was isolated and quantitative RT-PCR was performed to determine the levels of PSA (A) or hK2 (B) mRNA. Data shown are representative of two similar experiments.

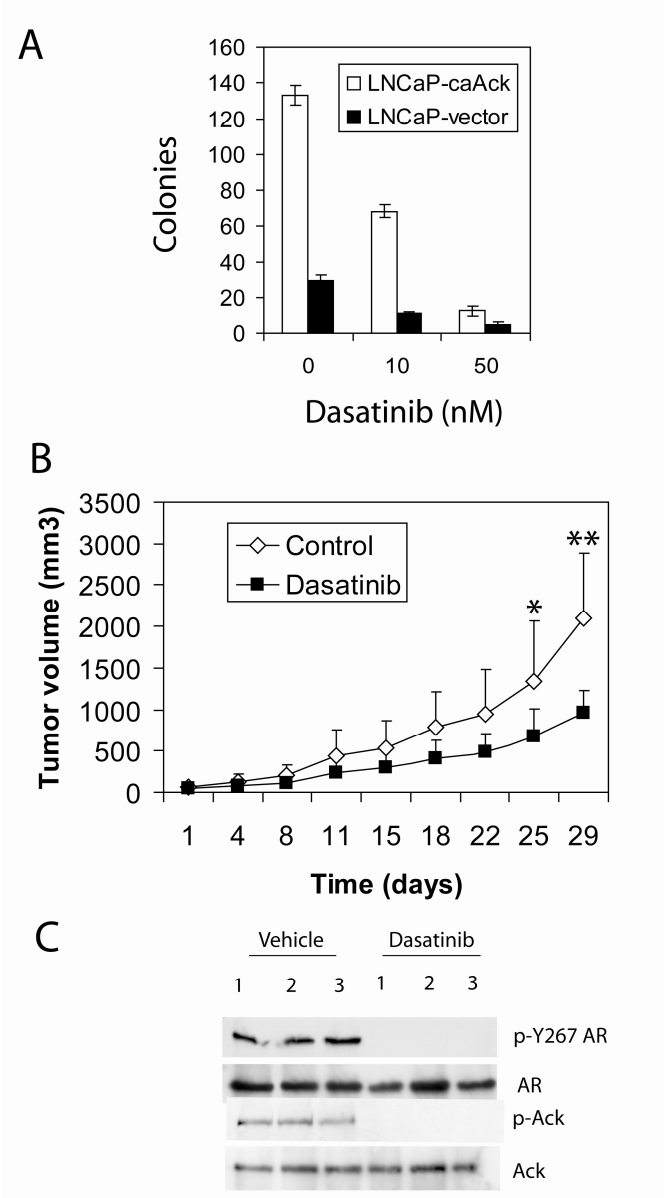


**Figure 2.10. Dasatinib inhibits anchorage-independent colony formation and xenograft tumor growth of LNCaP-Ack1 cells in castrated mice.** (A) LNCaP-Ack1 cells and vector control cells were plated in soft agar with indicated concentrations of dasatinib in the top layer and incubated for 21 days. Colonies were stained and counted. The mean and standard deviation of triplicate wells of a representative experiment are shown from three independent experiments with similar results. (B) Castrated mice were implanted subcutaneously with LNCaP-Ack1 cells. When tumors became palpable, mice were treated with vehicle control (n=7) or dasatinib (n=8) via oral gavage at a dose of 30 mg/kg bid and the tumor volume was measured twice per week. (* indicates $p<0.04$ and ** indicates $p<0.009$.) (C) Three independent tumors were harvested from mice given vehicle only or dasatinib at the conclusion of the experiment. Protein extracts were immunoblotted for expression of total Ack1, phospho-Ack1, total AR and phospho-Y267 AR.

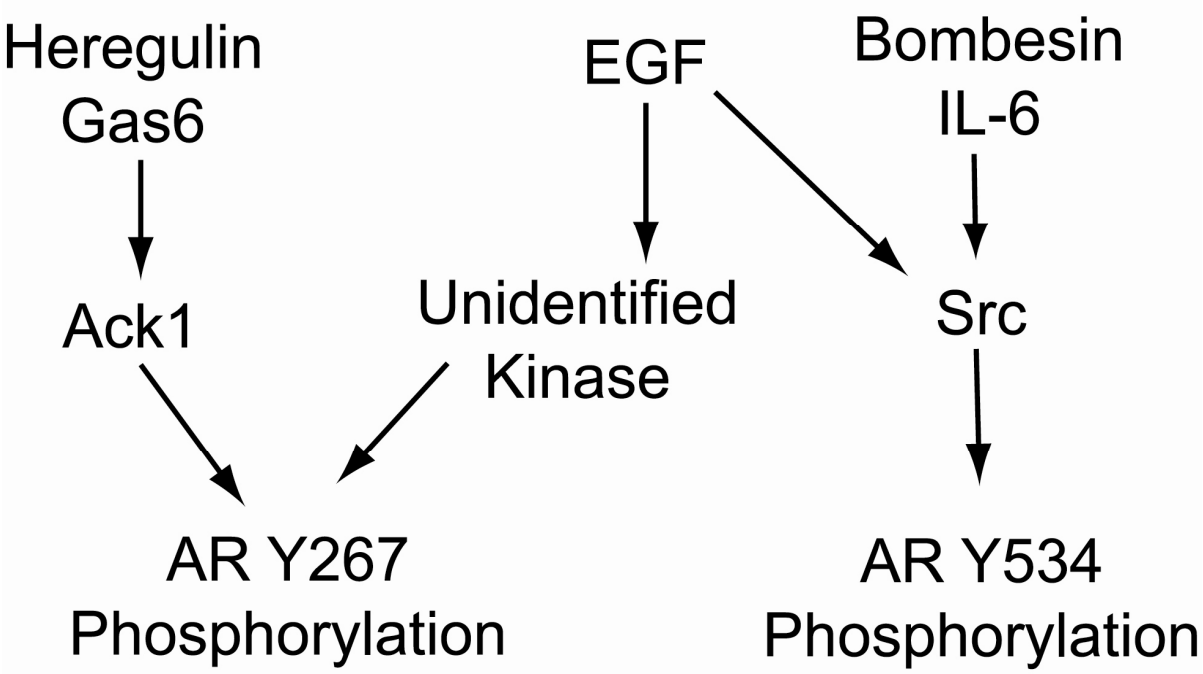


**Figure 2.11. Model of AR phosphorylation by non-androgen ligands.** Heregulin and Gas6 activate Ack1 kinase, which leads to AR Tyr-267 phosphorylation. EGF, bombesin, and IL-6 activate Src kinase, leading to AR Tyr-534 phosphorylation. Additionally, EGF activates an unidentified dasatinib-resistant tyrosine kinase that phosphorylates AR at Tyr-267.

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# CHAPTER III

## REGULATION OF ANDROGEN RECEPTOR FUNCTION BY TYROSINE PHOSPHORYLATION

This chapter has been adapted from: Mehmet Karaca, Yuanbo Liu, Zhentao Zhang, Dinuka De Silva, H. Shelton Earp, and Young E. Whang. Regulation of androgen receptor by tyrosine phosphorylation. 2010. Manuscript in progress.

## 3.1 Abstract

The androgen receptor (AR) is a ligand activated steroid hormone receptor that plays a critical role in normal prostate development and prostate cancer. AR re-activation under low androgen environment is the hallmark of castration resistant or hormone refractory prostate cancer (HRPC). Non-receptor tyrosine kinases phosphorylate AR in an androgen independent manner, and are thereby involved in HRPC. Herein, we investigated the role of Ack1 target phosphorylation sites (Tyr-267 and Tyr-363) on growth factor-regulated AR phosphorylation as well as AR transcriptional and functional activity. Both Tyr-267 and Tyr-363 have a critical role for ligand-dependent and –independent control of activity of AR. Treatment of LNCaP cells overexpressing full length or truncated AR (missing the ligand binding domain (LBD) with epidermal growth factor (EGF), heregulin, or Gas6 (ligand binding to Mer receptor tyrosine kinase and activating Ack1 downstream) induced AR phosphorylation at Tyr-267 and that phosphorylation was lost in the AR-Y267F mutant protein. The full length wild type overexpressing cells (FL-WT-AR) proliferated strongly without androgen treatment and they reached optimal growth at a lower dose of DHT treatment (0.1 nM DHT). However, cells expressing full- or -truncated AR-Y267F mutant did not show androgen-independent proliferation and did not respond to androgen treatment. Overexpression of the Y267F mutant within full length- or truncated-AR showed significant reduction in soft agar colony formation, compared to the WT-AR. The extent of reduction in colony formation of Y363F AR expressing cells was moderate, but not as much as Y267F. The analysis of AR subcellular localization by both immunoblotting and immunofluoresence assays suggested that mutating the Tyr-267 site impaired both androgen-dependent and –independent nuclear translocation, compared to the AR-WT. Global gene expression

profiling analysis demonstrated that there were no common genes regulated by both full length AR and AR-Y267F, suggesting that mutating Tyr-267 has a significant impact on not only AR dependent target gene expression but also global gene expression of LNCaP cells. Taken together, the results of our study demonstrate that tyrosine kinase target phosphorylation sites are important for both ligand-dependent and ligand-independent activity of AR protein. Targeting upstream tyrosine kinases and the N terminal domain of AR in truncated AR missing LBD may expand the repertoire of therapeutics for combating prostate cancer.

## 3.2 Introduction

Prostate cancer is the most commonly diagnosed cancer (192,280 new cases each year) and the second leading cause of death (27,360 deaths annually) among US men (Jemal et al., 2009). Hormone refractory prostate cancer (HRPC) develops after the systemic hormone deprivation therapy of initially hormone dependent prostate cancer (Armstrong and Carducci, 2006; Isaacs and Isaacs, 2004). The exact mechanisms by which hormone dependent prostate cancers evolves into hormone refractory disease remain to be solved, yet there is emerging consensus indicating that re-activation of androgen receptor is a common phenomenon in HRPC (Agoulnik and Weigel, 2006; Chen et al., 2004; Scher and Sawyers, 2005). Recent reports showed that there is a number of ways that AR become activated in the low androgen environment (Feldman and Feldman, 2001; Scher and Sawyers, 2005). For example, ligand independent AR reactivation by growth factors such as epidermal growth factor or heregulin is one of the mechanism by which prostate cancer cells sustain AR activity. Likewise, AR gene amplification or mutations in the ligand binding domain due to hormone deprivation therapy makes prostate cancer cells sensitized to low androgen levels

and broaden ligand specificity of AR, respectively (Ford et al., 2003; Linja et al., 2001; Visakorpi et al., 1995; Wallen et al., 1999; Waltering et al., 2009). AR protein overexpression is commonly detected in clinical HRPC (Latil et al., 2001; Linja et al., 2001). Comparative gene expression analysis of hormone dependent and refractory human prostate cancer xenograft tumors identified AR as the only gene overexpressed in hormone refractory xenografts (Chen et al., 2004). Furthermore, co-activators such as TIF-2 or SRC1 were upregulated in hormone refractory prostate cancer (Gregory et al., 2001). Growth factors such as EGF or heregulin activate the AR under androgen deprivation therapy (ADT) in prostate cancer (Gregory et al., 2004). The increase in AR stability along with AR overexpression and nuclear localization render prostate cancer cells to become very sensitive to low levels of androgen present in androgen deprived conditions (Gregory et al., 2001).

The modulation of AR signaling pathway though tyrosine phosphorylation of AR by non-receptor tyrosine kinases is becoming a common theme among the mechanisms of AR activation and prostate cancer progression (Guo et al., 2006; Kraus et al., 2006; Mahajan et al., 2007). Non-androgen ligand induced non-receptor tyrosine kinases such as Src or Ack1 can directly phosphorylate and activate the AR in prostate cancer (Guo et al., 2006; Mahajan et al., 2007). Guo et al. (2006) showed that Src can activate AR and induce its nuclear translocation by phosphorylating AR at Tyr534. Likewise, Mahajan et al. (2007) showed that Ack1 phosphorylates AR at Tyr-267 and 363 sites and activates AR without androgen and transforms poorly tumorigenic androgen dependent LNCaP prostate cancer cell into castration recurrent highly tumorigenic cells in nude mice xenograft. Characterization of upstream signaling of these two kinases showed that Ack1 were activated by epidermal growth factor (EGF), heregulin, and Gas6 (ligand binding to Mer receptor tyrosine kinase

and activating Ack1 downstream). Src was activated by EGF, interleukin (IL)-6 or bombesin, respectively (Liu et al., 2010). The Ack1 activation led to AR phosphorylation at Tyr-267, however, Src activation induced phosphorylation of AR at Tyr-534. Dasatinib, a Src inhibitor, inhibited EGF induced AR phosphorylation at Tyr-534. Likewise, dasatinib treatment prevented heregulin mediated AR phosphorylation at Tyr-267.

In addition to these mechanisms, there are several recent reports that indicated that aberrant pre-mRNA splicing producing truncated constitutively active AR species that are resistant to anti-androgen or hormone deprivation therapy may be the underlying mechanism of how prostate cancer cells survive under ADT (Dehm et al., 2008; Dehm and Tindall, 2006; Hu et al., 2009; Libertini et al., 2007; Liu et al., 2009; Pelley et al., 2006). Libertini et al. (2007) showed that calpain mediated AR cleavage created an AR protein variant that is missing CTD and constitutively active in 22Rv1 cells that expresses both truncated AR isoforms and full length wild type AR (Libertini et al., 2007). They also reported elevated expression of the truncated AR isoforms in prostate tumor tissues. Likewise, Dehm et al. (2008) reported that the 22Rv1 cell line, derived from the relapsed CWR22 xenograft during ADT, expressed three isoforms of AR: a full length version with duplicated exon 3 (ARex3dup), and two truncated versions lacking the COOH terminal domain (CTD) (ARex1/2/2b or ARex1/2/3/2b) (Dehm et al., 2008). Unlike the reports of Tepper et al. (2002) and Libertini et al. (2007), Dehm et al. (2008) showed that truncated isoforms (ARex1/2/2b or ARex1/2/3/2b) of AR were not due to proteolytic cleavage off AR with exon 3 duplication (ARex3dup) but derived from distinct mRNA species (Libertini et al., 2007; Tepper et al., 2002). AR NTD isoforms expressed in 22Rv1 cells are the result of the splicing of a novel exon 2b after AR exon 2 (ARex1/2/2b) or exon 3 (ARex1/2/3/2b). Exon

2 encodes the first zinc finger that harbors the recognition helix that directly engages with one hexameric half site within an androgen response element (ARE) (Shaffer et al. 2004). Hu et al. (2009) identified number of splice variants of AR and developed variant specific antibody to screen prostate cancer samples (Hu et al., 2009). The variant encoding NTD of AR was highly expressed in HRPC and expression of AR-V7 at protein levels was found to be increased in hormone naïve PCa recurring after surgical therapy. Mutation induced truncated AR missing LBD has been reported as constitutively active (Ceraline et al., 2004). Ceraline et al. (2004) using a yeast based functional assay showed that AR-Q640Stop/T877A mutation isolated from bone marrow metastasis of HRPC was constitutively active in the absence of androgens. Quayle et al. (2007) showed that the decoy molecule derived from AR NTD inhibited growth of prostate cancer xenograft indicating that AR NTD species are driving force behind hormone refractory growth (Quayle et al., 2007). A comprehensive study done by Guo et al. (2009) reported that constitutively active AR splice variant AR3 is highly expressed in androgen independent cell lines (C-81, CWR-R1, and 22Rv1) and AR3 expression is inversely correlated with androgen dependence of the cell lines (Guo et al., 2009). The AR3 expression was not affected by androgen or anti-androgen therapy and seemed to be playing an indispensable role for androgen independent growth of prostate cancer cells. The AR3 protein level was higher in the hormone refractory prostate tumor samples compared to hormone naïve tumors or benign prostate hyperplasia. These reports suggest that prostate cancer evades the androgen deprivation therapy (ADT) and becomes HRPC due to the emergence of splice variants that are missing ligand binding domain and resistant to the anti-androgen and androgen deprivation therapy. It has been reported that prostate cancer cells in hormone refractory stages induced testosterone synthesis from

adrenalin androgens or cholesterol that is sufficient to activate the AR signaling pathway because of the presence of highly sensitized AR protein (i.e. overexpressed AR) (Titus et al., 2005).

The purpose of this study was to understand the role of Ack1 target tyrosine phosphorylation sites, Tyr-267 or Tyr-363, in AR activation and function under low androgen environment by generating full length or truncated AR, constitutively active and missing ligand binding domain, and stably overexpressing these expression constructs in LNCaP cell lines and analyzing their phenotype. We showed that mutating Tyr-267 site abrogated the transcriptional activity of AR, cell proliferation, and anchorage independent growth of LNCaP cells. The result of AR nuclear localization assays suggested that mutation of Tyr-267 impedes AR nuclear localization.

## 3.3 Materials and Methods

**Cell Lines and Reagents.** LNCaP, COS-7, HEK293 cells were obtained from the University of North Carolina Tissue Culture Facility. LNCaP cells were grown in phenol red free RPMI 1640 supplemented with 10% FBS and 100 unit/ml penicillin/streptomycin (P/S). Both COS-7 and HEK293 were grown in DMEM supplemented with 10% FBS and P/C. EGF, IL-6 and Gas6 were purchased from R&D Systems, Minneapolis, MN. Heregulin was a gift from Genentech (South San Francisco, CA)(Liu et al., 2010). Phospho-specific polyclonal antibody against Tyr-267 of AR was raised and purified by a commercial vendor ($21^{st}$ Century Biochemicals, Marlboro, MA). A mouse monoclonal antibody against AR (F39.4.1, Biogenex, San Ramon, CA) was used for the immunoblotting and immunofluorescence. The polyclonal antibody against AR (C-19, Santa Cruz) was used to immunoprecipitate AR from the protein lysate. A mouse monoclonal antibody against Flag-

tag (M2) (Sigma Life Sciences, St. Louis, MO) and Flag conjugated agarose beads (Sigma Life Sciences, St. Louis, MO) were used for immnuoblotting and immunoprecipitation, respectively. Rabbit polyclonal antibodies against Laminin A/C (H-110) and 14-3-3 β (c-20) (Santa Cruz Biotehnology Inc., Santa Cruz, CA) were used in immunoblotting of subcellular fractions. Donkey anti-mouse FITC labeled secondary antibody (Jackson ImmunoResearch Laboratories, Inc. West Grove, PA) and mounting media with DAPI were used for staining AR and nucleus, respectively.

**Western Blotting.** Cells were lysed in complete RIPA buffer supplemented with 1X protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN), and 1X phosphatase inhibitor cocktail 1 (Sigma-Aldrich, St. Louis, MO) and incubated on ice and centrifuged to clear the cell debris. The supernatant was collected and stored in -80 until western blotting. The small aliquots of proteins were quantified with the Bradford assay reagent (Bio-Rad Laboratories, Hercules, CA) and 20 µg of protein sample was resolved in 10% SDS PAGE gel for 2 hrs. Protein samples were transferred to the PVD membrane and subsequently blocked in 5 % non-fat dry milk in 1X TBS-T for one hour. The membrane was incubated with AR antibody (Biogenix Inc) in 5% non-fat dry milk in TBS-T overnight. Membranes were subsequently washed 10 minutes each three times with 1X TBS-T and incubated with anti-mouse secondary antibody for AR for 2 hours. After incubation, membranes were washed three times for 10 minutes each with 1X TBS-T and developed with ECL solutions (Amersham Inc.).

**Transfections.** COS7 and LNCaP cells were transiently transfected with either full length or truncated AR expression vectors using Effectene (Qiagen, Valencia, CA) or

FuGENE 6 (Roche Applied Science, Indianapolis, IN), following manufacturer's protocol. Three experimental replicates were done for each assay.

**Reporter Assays.** LNCaP cells were plated (1.5 X $10^5$ cells per well) in 12 well plates using their respective growth medium the day before transfection. Next day, 150 ng of the AR construct (wild type or mutant) and 150 ng of the reporter construct (ARR2-PB or MMTV-luciferase reporter) were co-transfected using Effectene. Next day, medium was replaced with phenol-red free, serum free media and incubated for 8 hours. Following serum starvation, the cells were treated with DHT (10 nM) or vehicle (1% Ethanol) in serum free media for overnight. The cells were harvested in passive lysis buffer and luciferase activity was measured on an Lmax Microplate Luminometer (Molecular Devices), as described (Mahajan et al., 2007).

**Colony Formation in Soft Agar.** LNCaP cells stably expressing vector, WT-AR, or mutant AR constructs ($10^4$ cells per well) were suspended in 0.45% Noble agar and placed above a layer of solidified 0.9% Noble agar in 6 well plates in triplicates. After 3 weeks, colonies were visualized by staining with MTT as described in (Liu et al., 2010).

**Subcellular Fractionation of Protein.** Subcellular fractionation of cells was performed by using NE-PER Nuclear and Cytoplasmic Extraction Reagent (Pierce Biotechnology Rockford, IL) supplemented with protease inhibitors to cytoplasmic extraction reagent I (CER I) and nuclear extraction reagent (NER) as described in their protocol. Both cytoplasmic and nuclear extracts were quantified with the Bradford assay reagent (Bio-Rad Laboratories, Hercules, CA) and an equal amount of protein was used in western blotting.

**Immunofluorescence.** Cells were counted and $3x10^4$ cells were plated in each well of two well chamber slides (LabTek II System, Nalge Nunc Int. Corp., Naperville, IL). Next day, cells were transiently co-transfected with AR (100 ng) or vector (100 ng) along with caAck (100 ng) or vector (100 ng) expression constructs using FuGENE 6 (Roche Applied Science, Indianapolis, IN) and incubated for overnight. Medium was changed to serum free medium and cells were serum starved for 8 hrs. After serum starvation, cells were either treated with DHT (10 nM) or vehicle (1% ethanol) for 2 hours. Next, media was removed from the cells and cells were rinsed with cold PBS once for 5 minutes. The fixative (4% paraformaldehyde) was added on cells and incubated at room temperature for 15 minutes. At the end of the fixation, cells were washed with cold PBS twice, and blocking solution (0.3% Triton X and 5% donkey serum) was added and incubated for 30 minutes at room temperature. At the end of the incubation, blocking solutions were removed from the wells except the blank samples. Primary antibody, mouse anti-androgen receptor (F39.4.1, Biogenex, San Ramon, CA) made in 1:100 dilution in blocking solution, was added on the cells and incubated in room temperature for two hour. At the end of incubation, primary antibody was removed from cells and cells were washed three times with cold PBS (5 minutes each wash). Secondary antibody, fluorescein isothiocyanate conjugated (FITC) donkey anti mouse, was prepared in 1:250 dilution in blocking solution and added to cells. The cells were incubated in dark in 37ºC for an hour. The secondary antibody was removed and cells were washed with cold PBS three times. At the end of the wash, cover slips were placed with mounting media which includes 4',6-diamidino-2-phenylindole (DAPI) for nuclear staining (Vector Laboratories Inc. Burlingame, CA). After cover slips were

mounted, the edges of the slides were sealed off with nail polisher and slides were kept in dark cold room until images were captured.

**Microarray Gene Expression Analysis.** Total RNA from LNCaP cells expressing vector, FL-WT-AR, FL-AR-Y267F, TR-WT-AR and TR-AR-Y267F were isolated by using RNeasy Mini kit (Qiagen Inc. Valencia, CA) by following manufacturer's instructions. Two independent replicas were used per cell lines. Each sample was assayed versus LNCaP cells expressing vector (Reference sample). RNA integrity was evaluated using the RNA 6000 Nano LabChip kit and a Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA, USA) at the Genomic Core Facility in UNC. The samples were hybridized onto Whole Human Genome Oligonucleotide Microarray (Human 4X44 genes) by following manufacturer's protocol at the UNC Genomic Core Facility. The microarrays were scanned on an Agilent scanner and the raw data extracted and deposited into the UNC Microarray Database (UMD). Background corrected normalized (lowess protocol) individual channel values were used as a data value for comparing gene expressions from LNCaP-vector cells to LNCaP cells expressing one of the four AR constructs. Following filtering was done to retrieve the data from UMD: signal intensity at least units in both channels (Cy5 and Cy3) and genes at least one standard deviation apart in at least one of the 8 arrays. A total of 18729 genes passed the filtering criterion and used an input for the significant analysis of microarray (SAM) program (Tusher et al., 2001). We next performed two class unpaired comparison between vector and AR constructs to select the genes significantly up or down regulated. In our SAM analysis to identify the gene sets, we used constant delta value of 10 to get a false discovery rate of 5 %. The SAM gene list from vector versus FL-WT-AR and vector versus FL-AR-Y267F were compared and the number of genes regulated by FL-WT-AR and FL-AR-Y267F as well as

genes that were only regulated by FL-WT-AR or FL-Y267F were identified by using JMP Program version 7 software (SAS Institute Inc. Cary, NC) and presented as a Venn diagram.

**Statistical Methods.** SAS software version 9 (SAS Institute Inc. Cary, NC) was used for statistical analysis of data. ANOVA procedure of SAS was used to evaluate differences between group means. *P* values less than 0.05 were considered to be statistically significant.

## 3.4 Results

**Phosphorylation Site Mutants of AR Exhibit Impaired Transcriptional Function in Reporter Assays.** The transcriptional activity of full length AR with mutations in the Ack1 phosphorylation sites Tyr-267 and Tyr-363 was tested in LNCaP cells using the AR reporter MMTV-luciferase construct. The AR Y267F mutant demonstrated significant impairment of transcriptional activity while the Y363F mutant exhibited moderately decreased activity (Fig. 3.1). To assess the role of phosphorylation sites in ligand-independent AR activation, the Y267F and Y363F mutants of truncated AR missing the ligand binding domain and constitutively active without ligand were tested by reporter assays using ARR2-PB and MMTV promoter constructs. The Y267F mutant of truncated AR exhibited marked reduction in its ability to activate the ARR2-PB and MMTV reporters. The Y363F mutant showed only minimally decreased transcriptional activity, relative to the wild type truncated AR (Fig. 3.2). Immunoblotting confirmed that protein levels of Y267F and Y363F mutants of full length and truncated AR were similar to wild type AR (Fig. 3.2). Taken together, these results suggest that both Tyr-267 and Tyr-363 sites are essential for both ligand-dependent and -independent transcriptional activity of AR, and that loss of Tyr-267 exhibits more severe impairment of transcriptional function, compared to Tyr-363.

**Stable Overexpression of Full Length AR and Constitutively Active Truncated AR and Phosphorylation Site Mutants.** The functional requirement for Tyr-267 and Tyr-363 sites of AR in driving cell proliferation at suboptimal androgen concentrations was investigated by stable overexpression of wild type and mutant AR constructs in the context of full length and truncated AR. The total protein levels of full length AR in cells stably expressing exogenous AR by retroviral transduction was 2-3 fold higher than endogenous AR in vector control cells (Fig. 3.3A). Levels of wild type, Y267F, and Y363F of truncated AR migrating at about 80 kDa were comparable to the endogenous AR (Fig. 3.3C). Detecting the exogenous AR by the epitope tag verified that wild type, Y267F, and Y363F AR were all expressed at comparable levels (Fig. 3.3A). AR mRNA levels in stably overexpressing cells were 3-4 folds higher than vector control cells in full length AR-expressing cells and 2-3 folds higher in truncated AR-expressing cells (Fig 3.3B and D). Immunoblotting with the phospho-Y267 specific antibody demonstrated that both full length and truncated exogenous AR proteins were phosphorylated at Tyr-267 after stimulation with EGF, heregulin, or Gas6 ligands, as previously shown for full length AR protein (Liu et al., 2010) and that phosphorylation of Tyr-267 is lost in Y267F AR protein (Fig 3.4). Mutation of Tyr-363 did not affect phosphorylation of Tyr-267.

**Phosphorylation-site mutants of AR are defective in stimulating cell proliferation.** The effect of AR overexpression on cell proliferation at various androgen concentrations was characterized. Proliferation of vector control cells was androgen-dependent and required the full dose (10 nM) of DHT for optimal proliferation (Fig. 3.5A). Full length AR wt overexpressing cells proliferated well without androgen treatment and exhibited optimal proliferation to treatment with 0.1 nM DHT, which does not stimulate

proliferation of vector control cells (Fig. 3.5A). However, Y267F mutant AR-expressing cells did not proliferate in the absence of androgen or responded to increasing concentrations of androgen. Proliferation of Y363F mutant AR-expressing cells was intermediate between vector control and AR wt cells in the absence of androgen and at 0.1 nM DHT and did not respond to increasing androgen concentrations (Fig 3.5A). Cells expressing constitutively active truncated AR proliferated optimally without androgen (Fig. 3.5B). However, cells expressing the truncated AR with the Y267F mutation did not show androgen-independent proliferation and did not respond to androgen treatment. Cells expressing the truncated AR with the Y363F mutation showed modest reduction in androgen-independent proliferation, compared to AR wt and androgen treatment did not affect its growth (Fig. 3.5B). Collectively, these data suggest that the Tyr-267 and Tyr-363 sites are both critical for the function of overexpressed AR protein in stimulating cell proliferation at low androgen concentrations.

**Phosphorylation-site mutants of AR are defective in stimulating anchorage-independent soft agar colony growth.** The effect of AR overexpression and the effect of phosphorylation site mutation on anchorage-independent growth in soft agar were determined for both full length and truncated AR. Overexpression of full length or truncated AR wt significantly increased soft agar colony formation, compared to vector control cells (Fig 3.6A and B). Overexpression of the Y267F mutant of full length AR or truncated AR demonstrated marked reduction in soft agar colony formation, compared to AR wt (Fig 3.6A and B). The Y363F AR-expressing showed moderately decreased soft agar colonies, but not as much as the Y267F mutant. These results suggest that the ability of AR to enhance cell proliferation at low androgen concentrations and to stimulate anchorage-independent growth

in LNCaP cells requires intact phosphorylation sites Tyr-267 and Tyr-363. Mutation of Tyr-267 exhibits more severe impairment than Tyr-363 in these assays.

**Ack1 induced AR translocation into the nucleus in the absence of DHT.** AR is a ligand induced transcription factor that translocates into the nucleus and bind to the promoter or enhancer regions of AR dependent genes. Guo et al. reported that AR nuclear translocation was induced by phosphorylation of AR at Tyr-534 site by Src (Guo et al., 2006). We investigated the effect of Ack1 kinase on subcellular localization of AR in COS-7 cells by using transient transfection and immunofluoresence. COS-7 cells were transiently co-transfected with vector or Ack1 and AR constructs with or without DHT treatments for 2 hrs. Cytoplasmic and nuclear protein isolates were probed with monoclonal AR antibody. Without DHT treatment, AR localized mostly in the cytoplasm for vector co-transfected cells. However, caAck1 induced AR nuclear localization (Fig. 3.7A). In both vector and caAck1 co-transfected with full length wild type AR, there was nuclear localization in response to DHT treatment (Fig. 3.7A). For the FL-AR-Y267F and caAck1 co-transfected cells, AR mostly localized in the cytoplasm and DHT treatment induced nuclear localization was not as robust as that of FL-WT-AR. The nuclear localization pattern of FL-AR-Y363F was very similar to wild type in that AR was localized to the nucleus in response to caAck1 induction without androgen treatment and the amount of nuclear localization increased additively with androgen treatment of caAck1 co-transfected with FL-AR-Y363F (Fig. 3.7A). The result suggested that caAck1 induced androgen independent nuclear localization of AR protein, and mutating Tyr-267 inhibited caAck1 induced nuclear translocation. The immunofluoresence assay result also showed that Ack1-induced nuclear localization of AR was impaired by mutating Tyr-267 of AR, suggesting that Tyr-267 is essential for Ack1

induced AR nuclear translocation (Fig. 3.8). The effect of Ack1 target tyrosine phosphorylation sites on androgen- independent AR nuclear localization was evaluated in COS-7 cells transiently transfected with truncated AR expression constructs. The androgen-independent nuclear localization of constitutively active truncated AR was inhibited by mutating the Tyr-267 site. The effect of mutating Tyr-363 was not dramatic compared to TR-AR-Y267F (Fig. 3.7B). It has been previously reported that Src phosphorylates AR at Tyr-534 and induces its nuclear localization and its transcriptional activity. The result of our study suggested that Ack1 also phosphorylates AR at Tyr-267, similar to AR activation by Src, and induces the nuclear translocation and subsequent transcriptional activity of AR. The AR is a ligand induced transcription factor that gets into the nucleus and scans for the promoter and enhancer regions of the AR target genes and regulates their transcriptional activity. The defect in nuclear localization will impede the ability of AR to regulate target gene expression that might be involved in tumor progression.

**Androgen receptor overexpression leads to androgen-independent AR nuclear translocation.** In order to determine the role of AR overexpression on AR nuclear localization, we investigated subcellular localization of exogenous AR protein in LNCaP cells by immunoprecipitation with FLAG antibody and subsequently immunoblotting with AR antibody. Overexpression of both full length and truncated AR wt induced predominantly nuclear localization of AR without DHT treatment, yet overexpression of the Y267F mutant of full length and truncated AR predominantly localized in the cytoplasm and did not respond to the DHT induced nuclear localization observed with WT AR (Fig. 3.9A). The nuclear localization pattern of TR-AR-Y363F was very similar to wild type in that the

extent of impaired nuclear localization was not as dramatic as that of TR-AR-Y267F (Fig 3.9B).

**Y267F mutation has an impact on global gene expression.** The complex mechanism of hormone refractory prostate cancer progression cannot be fully explained by changes in a few genes. Therefore, it is essential to employ approaches that interrogate whole genome such as microarray analysis of gene expression. We studied the effect of mutating Tyr267 on global gene expression changes to characterize the defects we observed in transcriptional activity, proliferation and anchorage independent growth of LNCaP cells overexpressing Y267F mutant AR. The comparative analysis of samples with their respective vector references showed that the genes modulated by FL-AR-Y267F were very different from that of FL-WT-AR (Fig. 3.10A). In fact, there were no common genes that were regulated by both groups. However, comparison of TR-WT-AR and TR-AR-Y267F resulted in 50 genes that were regulated by both groups suggested that the defect in global gene expression modulation observed with mutating Tyr-267 was not as remarkable in the truncated, constitutively active AR context as the full length AR context (Fig. 3.10B). The comparison of gene expression changes in FL-WT-AR overexpressing cells with TR-WT-AR expressing cells suggested that 58 genes were commonly regulated by both AR constructs (Fig. 3.10C). It has been known that AR overexpression by itself has been sufficient to induce hormone refractory progression of androgen dependent prostate cancer cell (Chen et al., 2004). Likewise, recent reports suggested that AR splice variants constitutively active and missing the ligand binding domain were commonly observed in hormone refractory metastatic tumors (Guo et al., 2009). Our comparison of gene expression modulations in LNCaP cells overexpressing FL-WT-AR and TR-WT-AR will help to understand the some

of the underlying genetic changes occurring during these two different modes of AR regulated hormone refractory progression. The unsupervised clustering of samples based on gene expressions of 18,729 showed that experimental replica samples tightly clustered with each other suggested that repeatability of each of these two pairs of samples was high (Fig. 3.10D). In fact, we used intraclass correlation (ICC) procedure implemented in UMD to assess the array quality of each replica (Table A1.8). Interestingly, FL-WT-AR and TR-WT-AR were distantly clustered from each other reflecting the biological nature of the difference in full and truncated constitutively active AR (Fig. 3.10D).

**Y267F mutation inhibits recruitment and DNA binding of truncated AR to the androgen responsive enhancers of target genes.** To characterize the effect of Y267F and Y363F mutations of truncated AR in the chromatin context, recruitment and DNA binding of ectopically expressed FLAG-tagged truncated AR mutants to the PSA and HK2 androgen responsive enhancers were determined by chromatin immunoprecipitation using FLAG antibody (Fig 3.11A and B). Constitutively active TR-WT-AR protein was bound to the PSA and HK2 enhancers in the absence of androgen. The Y267F mutant did not bind to the PSA and HK2 enhancers (Fig. 3.11A and B). The Y363F mutant also showed diminished binding although its effect is not as dramatic as that of Y267F. Together, these results suggested that the Tyr-267 site has a critical role in the recruitment and DNA binding of constitutively active truncated AR missing LBD.

## 3.5 Discussion

Androgen receptor function is essential for both prostate cancer development and progression to hormone refractory disease. In addition to the mechanisms of AR activation studied so far, recent publications underlines the novel mechanisms of AR activation under

ADT including non-receptor tyrosine kinases and alternative splice variants of AR. Guo et al. (2006) and Mahajan et al. (2007) independently showed that two distinct tyrosine kinases Src and Ack1 targeted two distinct tyrosine phosphorylation sites with a similar outcome of AR activation and progression to hormone independent cell growth, proliferation and tumorigenesis. Recent work from our group suggested that growth factors such as EGF, heregulin, and Gas6 activated Ack1 and induced AR tyrosine phosphorylation at Tyr267. However, EGF, bombesin, and IL-6 induced Src activation and Tyr-534 phosphorylation of AR (Liu et al., 2010). The Src inhibitor, dasatinib, inhibited heregulin and Gas6 mediated Ack1 activation and Try-267 phosphorylation of AR at a clinically relevant dose. Here, we show that growth factor induced phosphorylation and activation of truncated AR were impaired by mutating AR-Y267F suggesting a novel mechanism of regulation of constitutively active AR species. The analysis of subcellular localization of both full and truncated AR expression vector in transient and stable expression settings suggested that mutating Tyr-267 had a defect in nuclear localization compared to WT-AR.

Another group of researchers elucidated the existence of constitutively active AR missing LBD (Dehm et al., 2008; Guo et al., 2009; Hu et al., 2009) which allowed prostate cancer cells to proliferate and grow aberrantly compared to the cells that did not express splice variants of AR. These two emerging mechanisms of activation of AR in HRPC led us to study the role of Ack1 target phosphorylation sites (Tyr-267 and Tyr-363) in the context of full length and truncated AR. We generated two groups of LNCaP cells lines overexpressing wild type and mutant AR expression vectors in the full and truncated context. In this report, we demonstrated that mutating Tyr-267 abrogated transcriptional activity, cell proliferation and anchorage independent growth observed with LNCaP cells overexpressing AR wt.

Our results showed that LNCaP cells over expressing FL- or TR-WT-AR mostly localized in the nucleus (Fig. 3.7 and 3.8) and intensity of nuclear localization increased in the FL-WT-AR expressing cells with androgen treatment (Fig 3.7A). The nuclear localization of truncated WT-AR did not seem to be affected by androgen treatment suggesting that under androgen depleted environment truncated AR variants can drive PCa growth and development. The defective nuclear localization of both FL- and TR-AR-Y267F mutant may be due to the impairment in interacting with nuclear importer signals, and therefore they cannot be transferred into the nucleus. This hypothesis should be further tested. Since the mutations we introduced (Tyr-267 and Tyr-363) are not in the region that encode nuclear localization signal (NLS) (Zhou et al., 1994), we did not expect the defect observed is due to the intrinsic NLS of AR that moves AR from the cytoplasm into the nucleus, but may be interacting with proteins that shuttles the AR into the nucleus.

Our results are consistent with the previous reports that suggested AR overexpression or kinase signaling pathways may make AR sensitized to the lower levels of androgen present in HRPC (Bakin et al., 2003; Culig and Bartsch, 2006; Hobisch et al., 2006). We showed that FL-WT-AR induced cell proliferation without androgen and maximum cell proliferation was observed at 0.1 nM DHT treatment compared to LNCaP cells over expressing vector that reached maximum growth at 10 nM DHT treatment (Fig. 3.4).

Both Ack1 induced AR activation and AR overexpression in LNCaP cells led to AR nuclear localization without androgen treatment (Fig 3.7A, 3.8 and Fig. 3.9). It has been known that nuclear receptors including AR shuttle between the cytoplasm and nucleus (Bunn et al., 2001; Dauvois et al., 1993; Guiochon-Mantel et al., 1996; Madan and DeFranco, 1993). There may be a defect in nuclear shuttling of mutant AR-Y267F that made AR-

Y267F mutant to localize predominantly in cytoplasm and to become insensitive to ligand-induced nuclear localization. If there is a defect in shuttling of AR, one way to address this problem is to use an approach such as heterokaryon shuttling assay (Black et al., 1999; Kesler et al., 2007) to elucidate whether the defect is in nuclear import of AR-Y267F.

Our results showed that overexpressing FL-WT-AR or TR-WT-AR was sufficient to induce cell proliferation and anchorage independent growth, suggesting that AR overexpression in different modes is enough to induce progression of hormone refractory disease, yet mutating AR phosphorylation sites abrogated the affect of over expression of AR suggesting that tyrosine phosphorylation of AR has a role in AR induced cell proliferation and anchorage independent growth.

Our results are consistent with the recent reports that identified novel splice variants missing LBD and highly expressed in hormone refractory prostate cancer (Guo et al., 2009; Hu et al., 2009), yet mutating Tyr-267 and Tyr-363 sites inhibits the strong transcriptional activity of AR NTD in both full length and truncated contexts. The Y267F mutant acted like a dominant negative that can even override the function of endogenous AR present in LNCaP cells. Since the phenotype observed with mutating Tyr-267 is stronger then that of mutating Tyr-363, we performed microarray gene expression profiling to characterize the global gene expression changes occurring when we mutated Tyr-267 compared to the AR wt within the full and truncated context. Microarray gene expression results suggested that FL-WT-AR and FL-AR-Y267F were two separate entities. There was not a overlapping gene list regulated by both expression constructs, yet TR-WT-AR and TR-AR-Y267F comparison led to a gene list that were modulated by both expression vector suggesting that defect in AR

activity due to loss of the Tyr-267 phosphorylation site was much dramatic in the full length context than truncated context.

Collectively, our results suggested that both full length and truncated AR overexpression in LNCaP cells is still modulated by AR tyrosine phosphorylation and subcellular localization. It has been know that Src can phosphorylate AR at Tyr-534 and induce nuclear translocation of AR under the androgen depleted condition (Guo et al., 2006). Likewise, growth factor induced AR phosphorylation at Tyr-267 may promote nuclear translocation and downstream transcriptional activity under the androgen deprived condition.

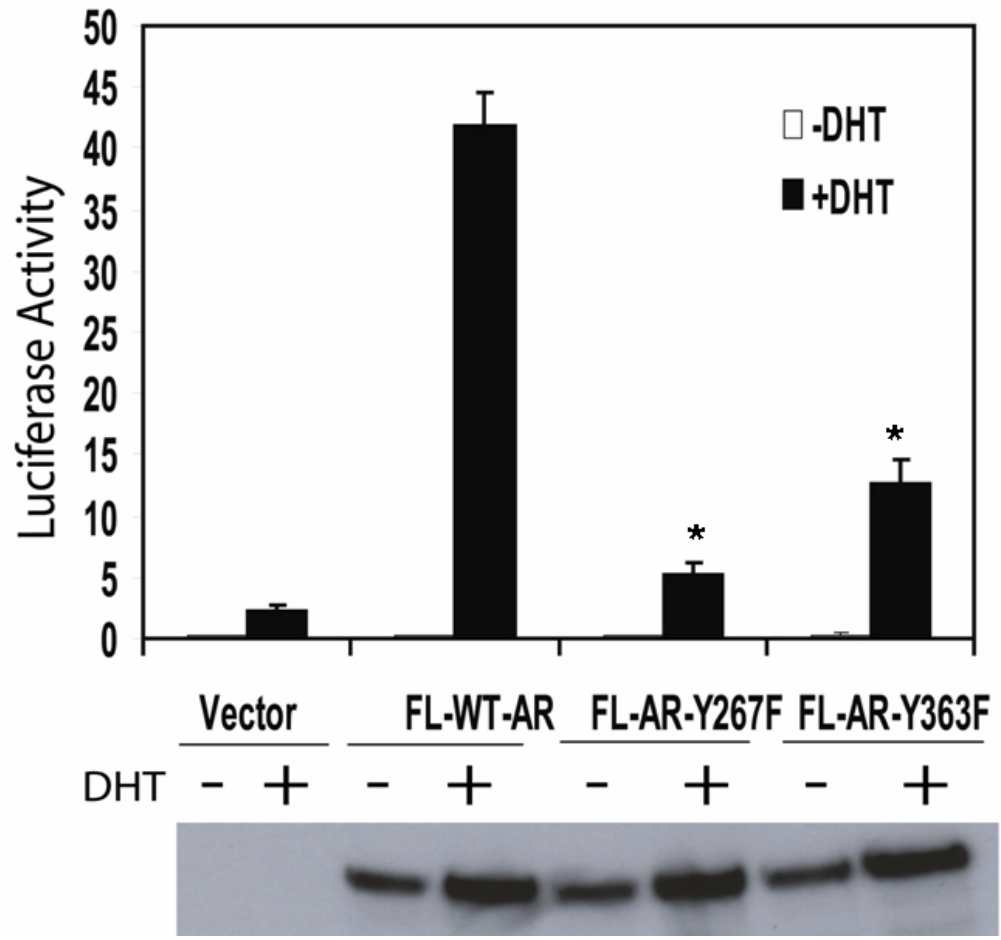


**Figure 3.1. Phosphorylation site mutants of full length AR exhibit impaired transcriptional function in reporter assays. Top panel.** LNCaP cells were transiently transfected with the expression vector encoding full length wild type AR (FL-WT-AR), Y267F or Y363F mutant along with the AR-dependent reporter MMTV-luciferase. Cells were treated with DHT (10 nM) for overnight (16 hrs). Luciferase activity was measured from cell lysates. The data shown with the mean and standard deviations of triplicate. Samples are representative of three independent experiments with similar results. **Bottom panel.** Cell lysates of COS-7 cells transiently transfected with respective AR constructs and treated with or without DHT overnight were immunoblotted with the monoclonal antibody against AR. Asterisk indicates statistical difference relative to FL-WT-AR ($P < 0.05$).

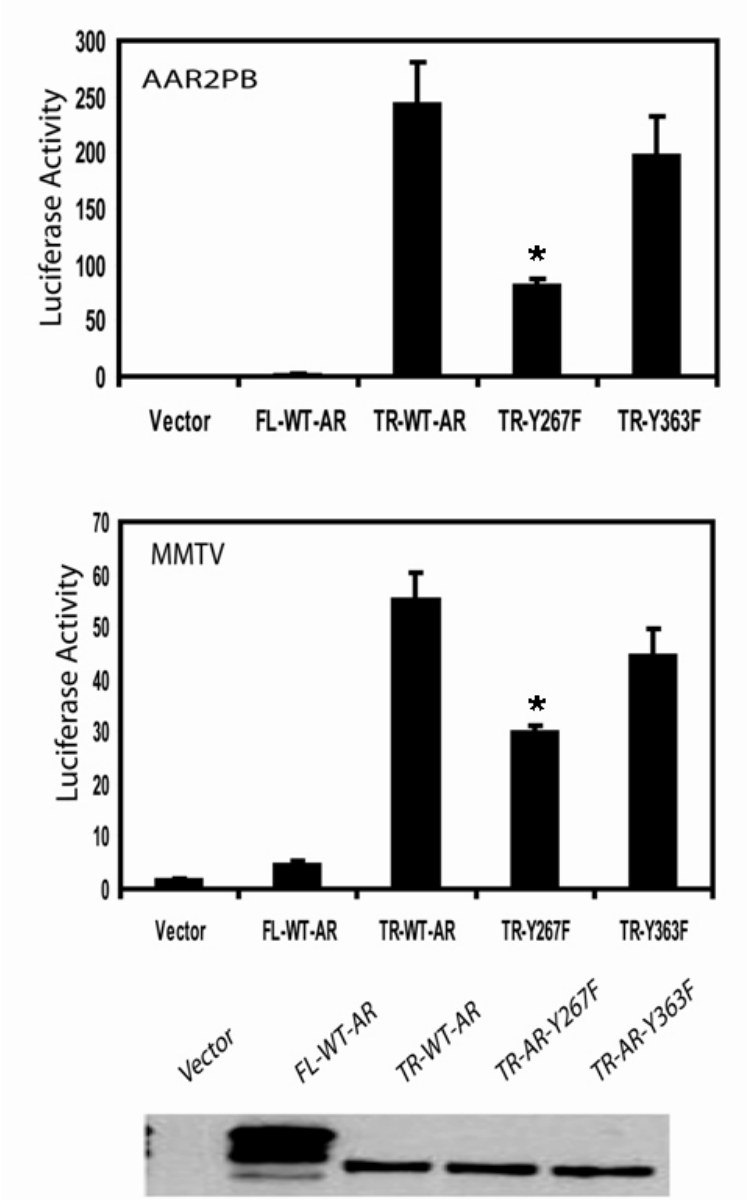


**Figure 3.2. Phosphorylation site mutants of truncated-AR exhibit impaired transcriptional function in reporter assays. Top panel.** LNCaP cells were transiently transfected with the expression vector encoding truncated constitutively active wild type AR (Tr-WT-AR), Y267F or Y363F mutant along with the AR-dependent report ARR2-PB-luciferase or MMTV-luciferase. After 48 hours, luciferase activity was measured from cell lysates. The data shows the mean and standard deviations of triplicates. Samples are representative of three independent experiments with similar results. **Bottom panel.** Cell lysates of COS-7 cells transiently transfected with respective AR constructs were immunoblotted with the monoclonal antibody against AR. Asterisk indicates statistical difference relative to TR-WT-AR ($P < 0.05$).

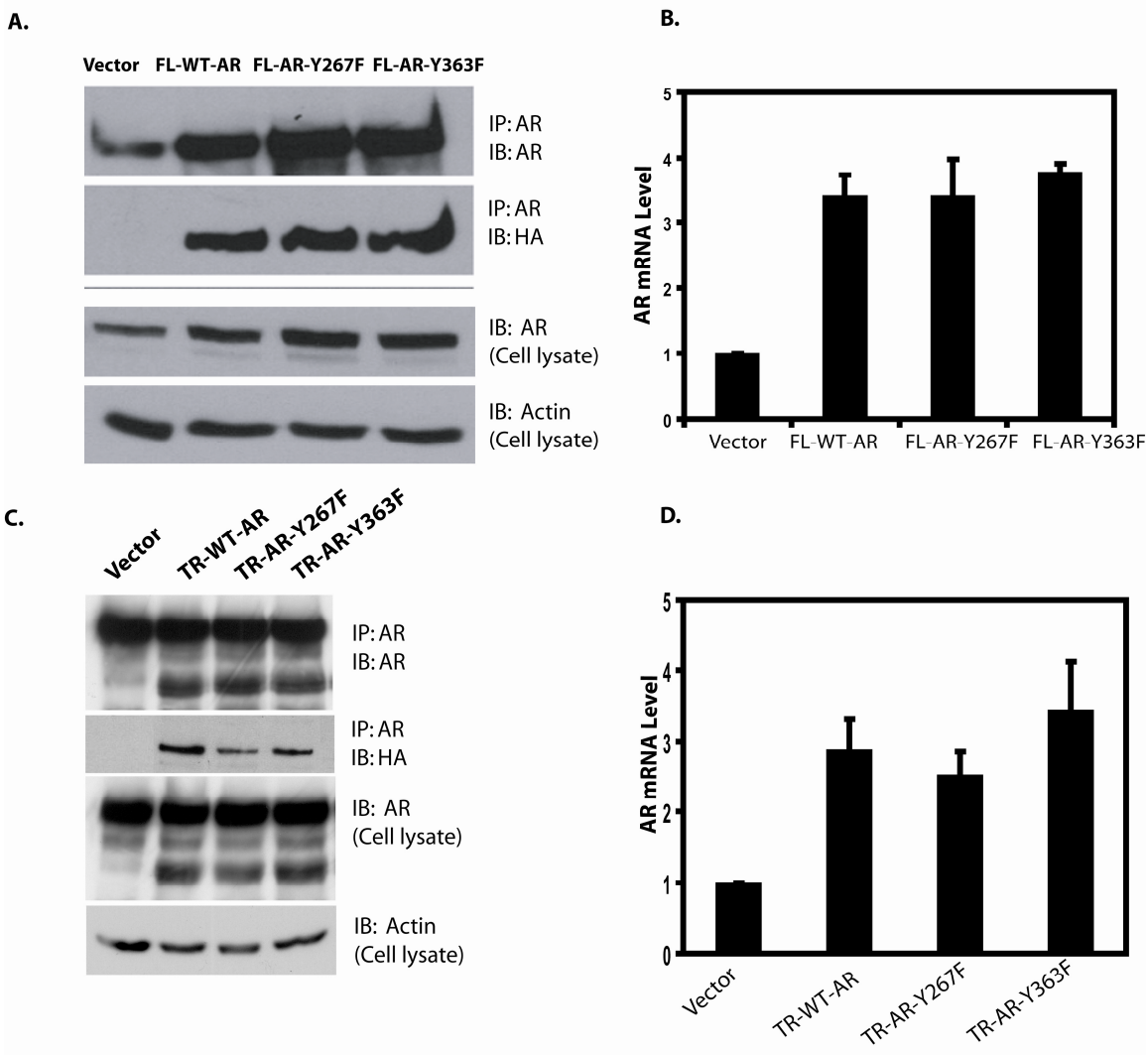


**Figure 3.3. LNCaP cells stably overexpress full length or truncated wildtype AR or phosphorylation site mutants.** LNCaP cells stably overexpressing AR constructs or vector control were derived by retrovirus-mediated transduction and antibiotic selection. **A.** Lysates from LNCaP cells overexpressing full-length AR were subjected to immunoprecipitation with AR antibody, followed by immunoblotted with AR or HA antibody, as indicated. Total cell lysates were also immunoblotted with AR monoclonal antibody and beta-actin antibody. **B.** The mRNA levels of AR was determined by quantitative reverse transcription PCR of total RNA isolated from LNCaP cells stably overexpressing full-length AR. **C.** Lysates from LNCaP cells stably overexpressing truncated AR were subjected to immunoprecipitation, followed by immunoblotting, as indicated. Total cell lysates were also immunoblotted with AR monoclonal antibody and beta-actin antibody. **D.** The mRNA levels of AR was determined by quantitative reverse transcription PCR of total RNA isolated from LNCaP cells stably overexpressing truncated AR.

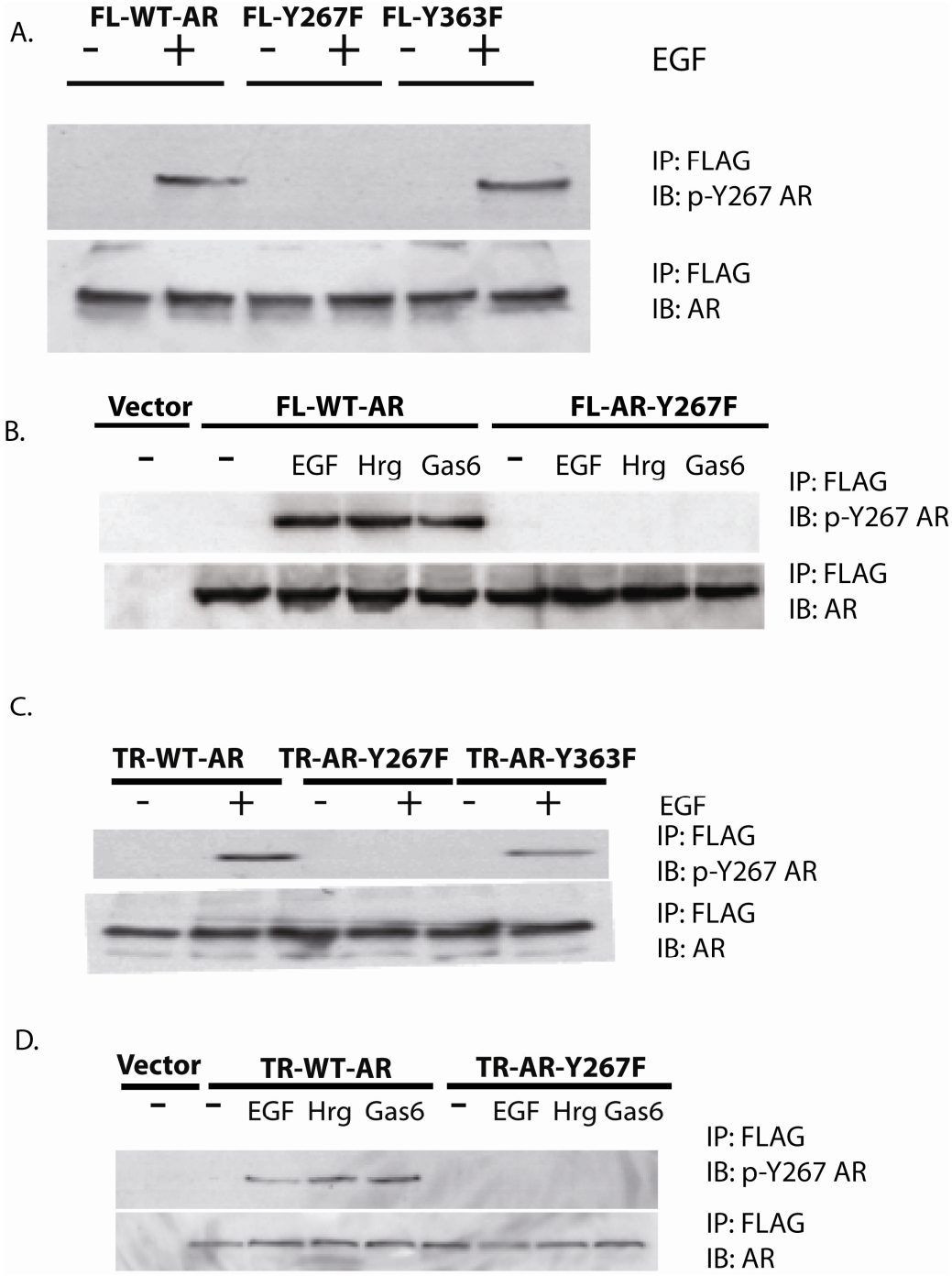


**Figure 3.4. The AR Y267F mutant protein stably expressed in LNCaP cells is not recognized by the phospho-specific antibody. A, B.** LNCaP cells stably overexpressing full length AR was treated with EGF (100 ng/ml) or heregulin (10 ng/ml) or Gas6 (100 ng/ml) for 1 hr, as indicated. Cell lysates were subjected to immunoprecipitation, followed by immunoblotting. **C, D.** LNCaP cells stably overexpressing truncated AR was treated with EGF (100 ng/ml) or heregulin (10 ng/ml) or Gas6 (100 ng/ml) for 1 hr, as indicated. Cell lysates were subjected to immunoprecipitation, followed by immunoblotting.

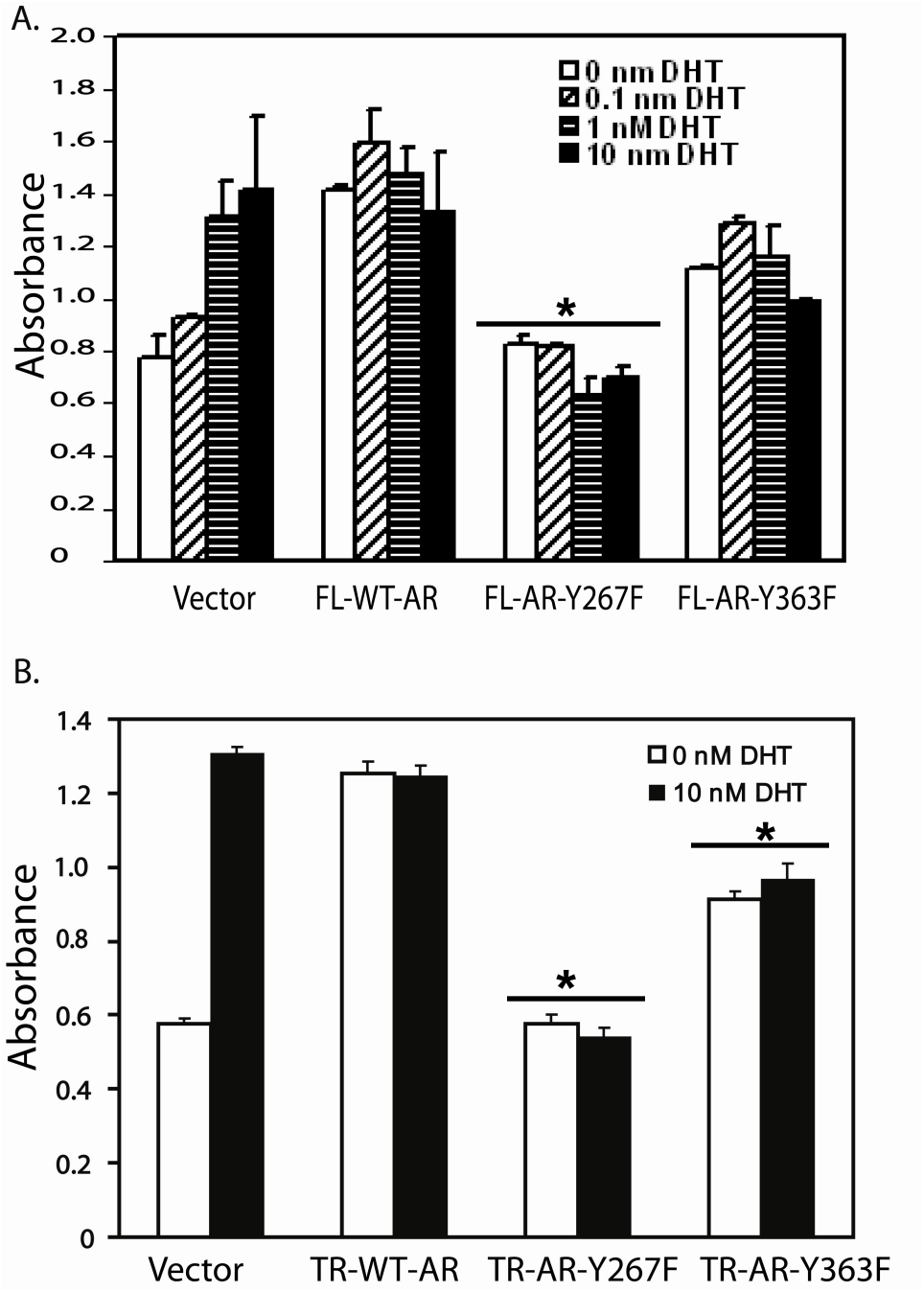


**Figure 3.5. The AR-Y267F mutant is defective in promoting cell proliferation A, B.** LNCaP cells stably overexpressing full length AR (A) or truncated AR (B) were grown in a 96-well plate for 72 hours in media with charcoal-stripped serum and indicated concentrations of DHT. The colorimetric dye WST-8 reagent was added and incubated for 4 hrs. Absorbance at 450nm was measured to determine relative cell proliferation. Asterisk and bar indicate statistical difference in corresponding doses relative to FL-WT-AR ($P < 0.05$) in (A), and TR-WT-AR ($P < 0.05$) in (B), respectively.

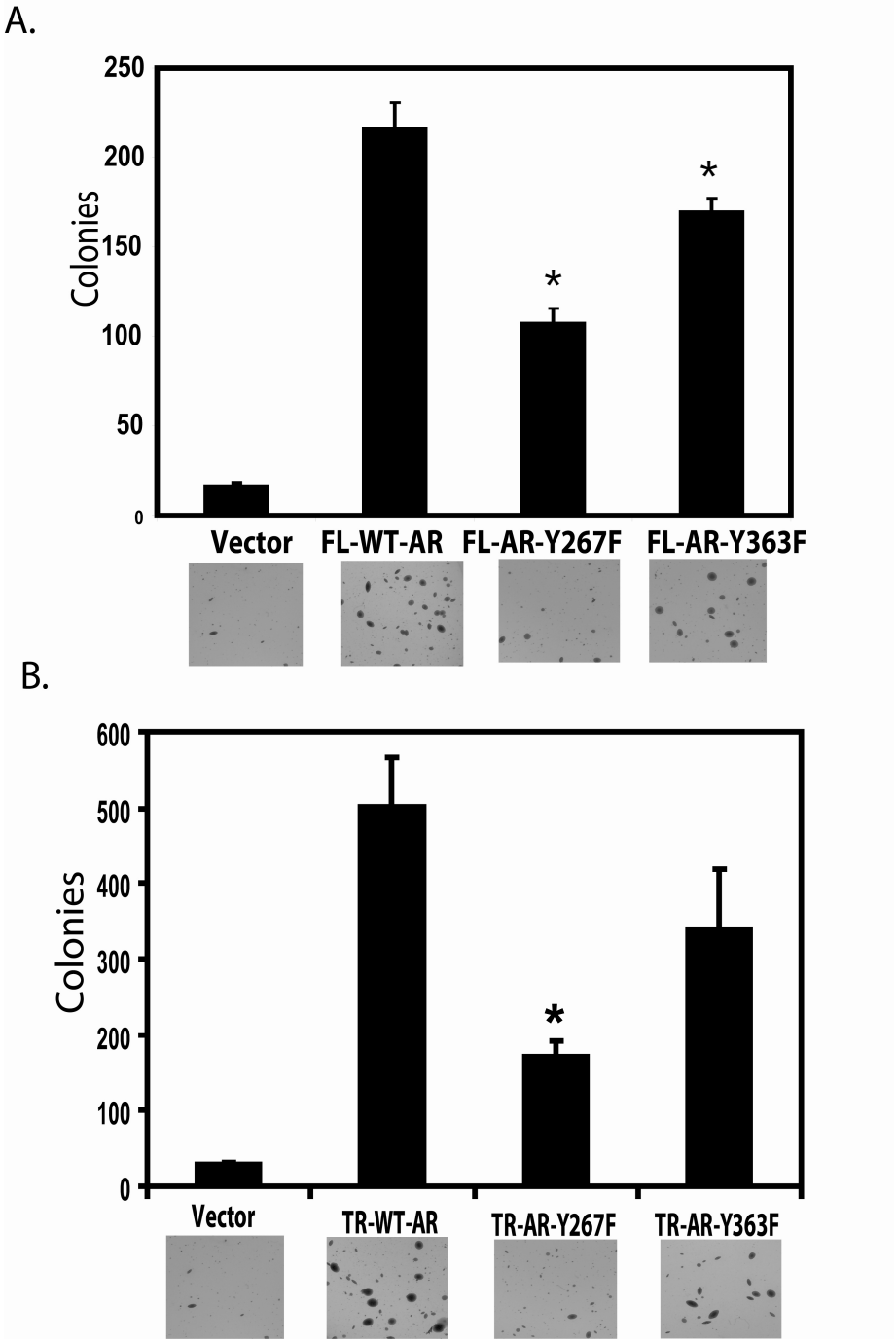


**Figure 3.6. The AR-Y267F mutant is defective in anchorage independent colony formation. A, B.** LNCaP cells stably overexpressing full length AR (A) or truncated AR (B) Cells were plated in soft agar and incubated for 3 weeks. Colonies were stained with crystal violet and counted. The data shown with the mean and standard deviation of triplicate wells are representative of three independent experiments with similar results. A representative view of the colonies is shown below the graph. Asterisk indicates statistical difference relative to FL-WT-AR ($P < 0.05$) in (A), and TR-WT-AR ($P < 0.05$) in (B), respectively.

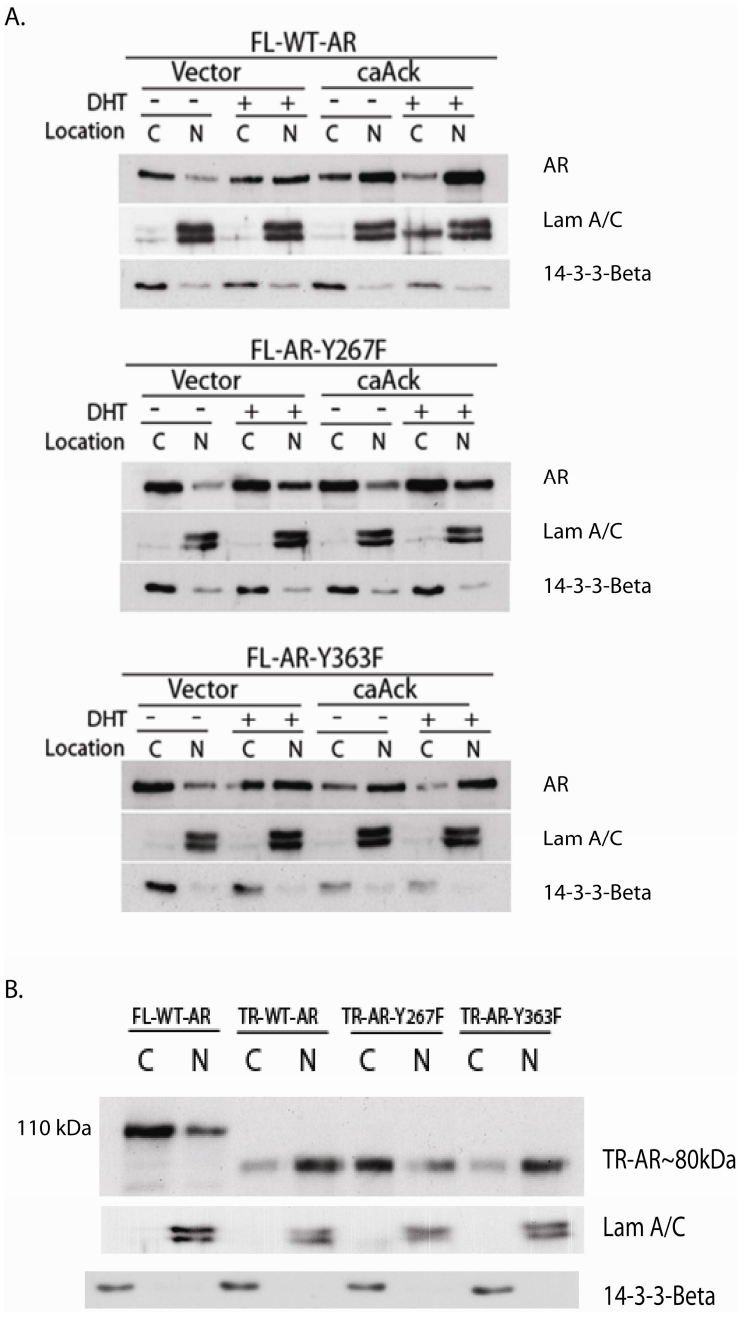


**Figure 3.7. The AR-Y267F mutant is defective in androgen- and Ack1-induced nuclear translocation. .** (A) and (B) COS-7 cells were co-transfected with the full length- or truncated-AR expression vector or truncated and the constitutively active Ack1 expression vector and incubated for 48 hrs. Then, the cells were serum-starved overnight and treated with or without 10 nM DHT for 2 hr. The cell fractionation was performed. Fifteen micrograms of protein from cytoplasmic (C) and nuclear (N) fraction were immunoblotted with AR antibody. Laminin A/C and 14-3-3 β were used as markers of nuclear and cytoplasmic fractions, respectively.

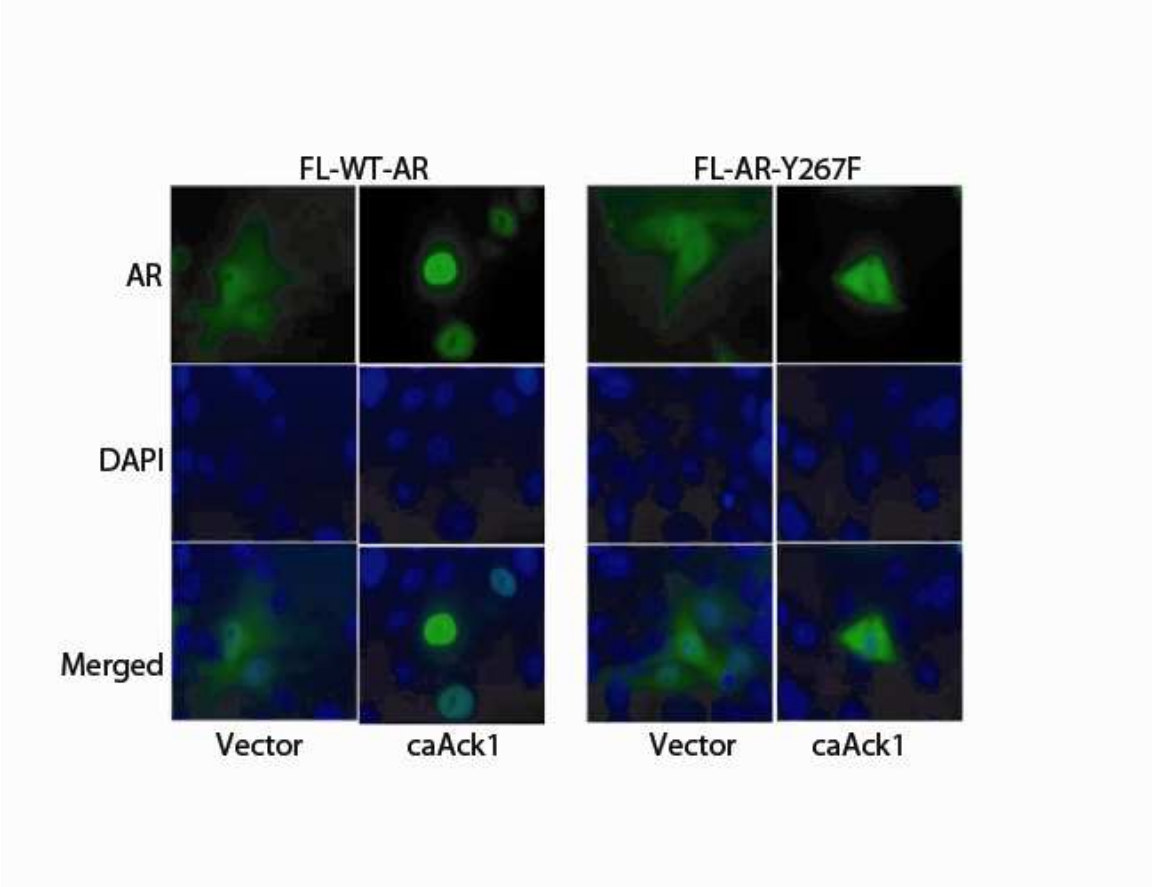


**Figure 3.8. The Tyr-267 is required Ack1-induced nuclear translocation of AR.** COS-7 cells were co-transfected with the AR expression vector and the constitutively active Ack1 expression vector and incubated for 48 hrs. Then, the cells were serum-starved overnight and treated with or without 10 nM DHT for 2 hr.

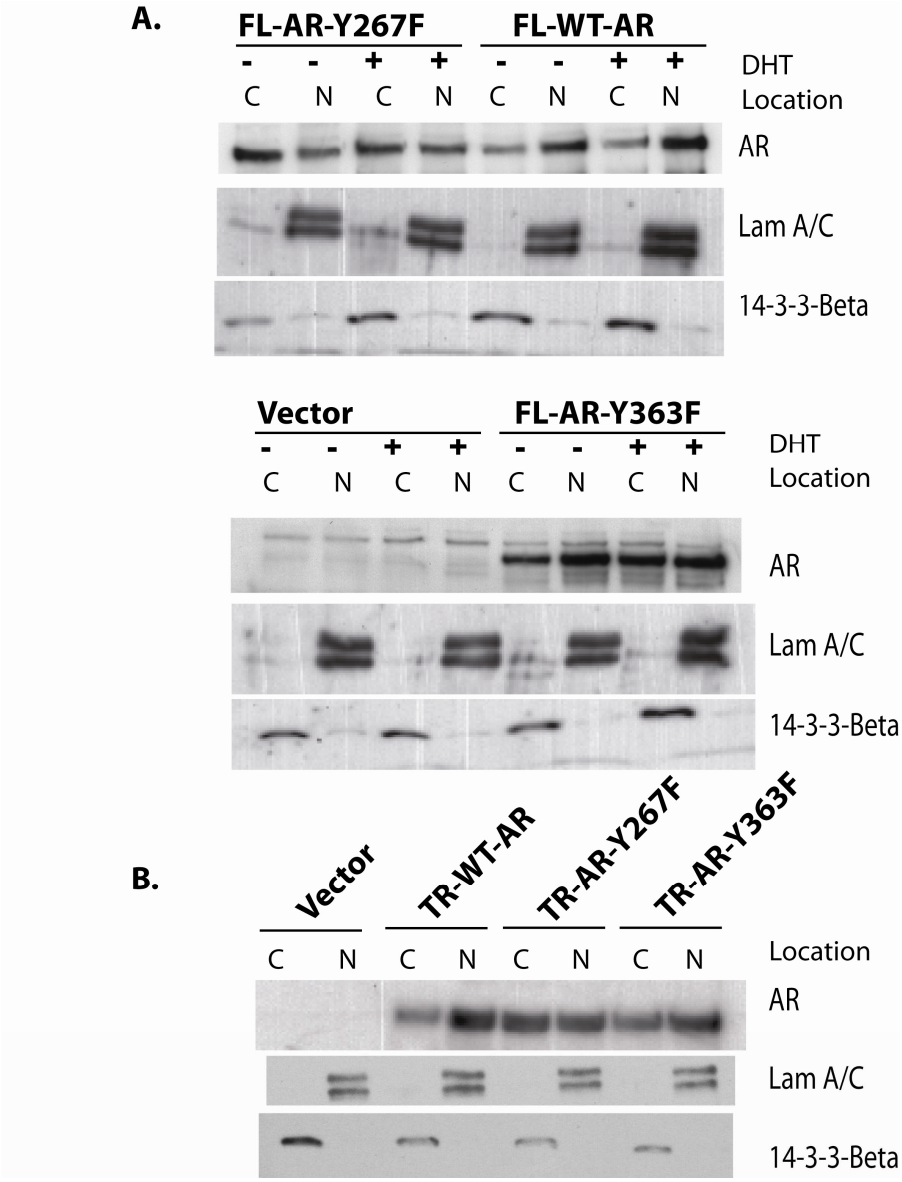


**Figure 3.9. The AR-Y267F mutant is defective in nuclear translocation in LNCaP cells stably overexpressing AR.** LNCaP cells stably overexpressing vector or AR constructs were grown in phenol red free media and serum starved for overnight and treated with or without 10 nM DHT for 2 hrs. The cell fractionation was performed. (A) The cytoplasmic and nuclear lysates from LNCaP cells overexpressing vector, FL-WT-AR, FL-AR-Y267F and FL-AR-Y363F were subjected to immunoprecipitation with Flag antibody, followed by immunoblotting with AR antibody as indicated. The total lysates were also immunoblotted with Laminin A/C and 14-3-3 β antibodies as markers of nuclear and cytoplasmic fractions, respectively. (B) The cytoplasmic and nuclear lysates from LNCaP cells overexpressing vector, TR-WT-AR, TR-AR-Y267F and TR-AR-Y363F were subjected to immunoprecipitation with Flag antibody, followed by immunoblotting with AR antibody as indicated. The total lysates were also immunoblotted with Laminin A/C and 14-3-3 β antibodies as markers of nuclear and cytoplasmic fractions, respectively.

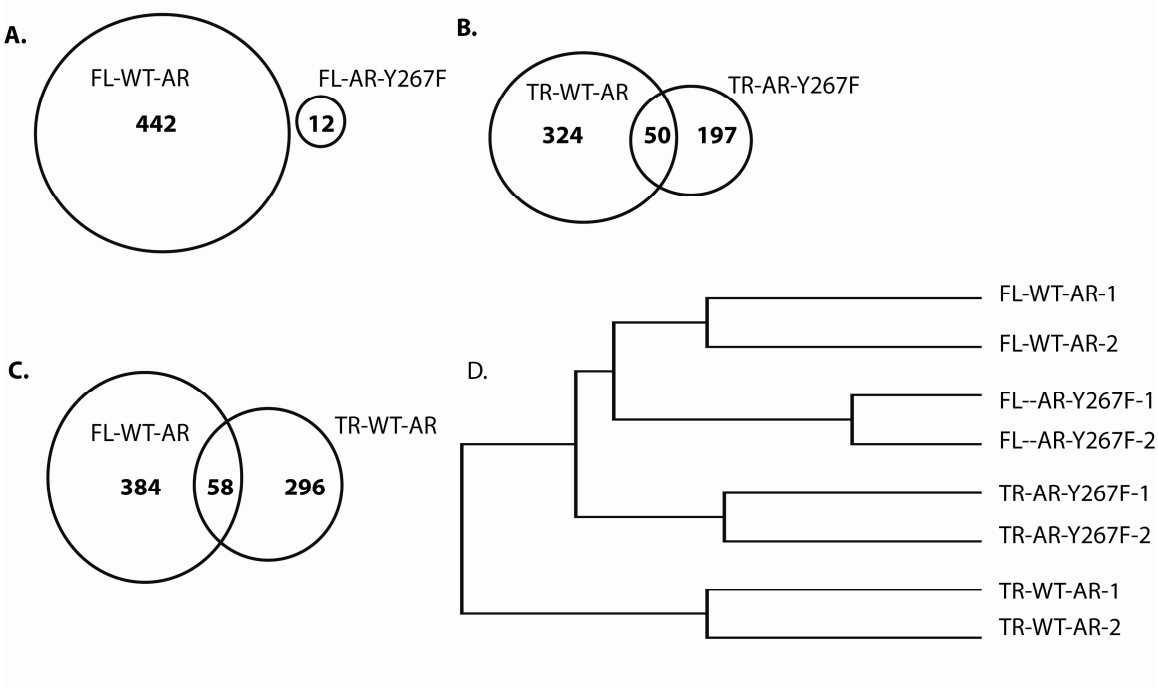


**Figure 3.10. Mutating the AR Tyr-267 site has an impact on global gene expression.** Background corrected, lowess normalized data values from each of the four pairs (FL-WT-AR, FL-AR-Y267F, TR-WT-AR, and TR-AR-Y267F) were retrieved from the UMD with the filtering procedure described in Materials and Methods. The data were consisted of 18729 genes. Two class unpaired comparison between vector and AR constructs were performed in SAM with constant delta ($\delta$=10) to have a false discover rate less than 5 % to identify genes differentially expressed between two groups. The SAM gene lists from AR constructs were compared to identify the gene modulated by individual constructs as well as both of the construct compared. The gene list comparisons were performed with JMP 7.1 and results were depicted as Venn diagrams. (A) The number of genes that were either modulated by FL-WT-AR or FL-AR-Y267F or both. (B) The number of genes that were either modulated by TR-WT-AR or TR-AR-Y267F or both. (C) The number of genes that were either modulated by FL-WT-AR or TR-WT-AR or both. (D) Experimental replicates clustered closely with each other. Unsupervised cluster analysis of eight samples was performed with 18,729 genes that passed the filtering criteria described in Material and Methods. Both genes and samples were clustered by using Cluster software (Eisen et al., 1998) with lowess normalized, median centered genes with average linkage method. The result of clustering was visualized with Java Treeview (Saldanha, 2004) and sample clusters were presented.

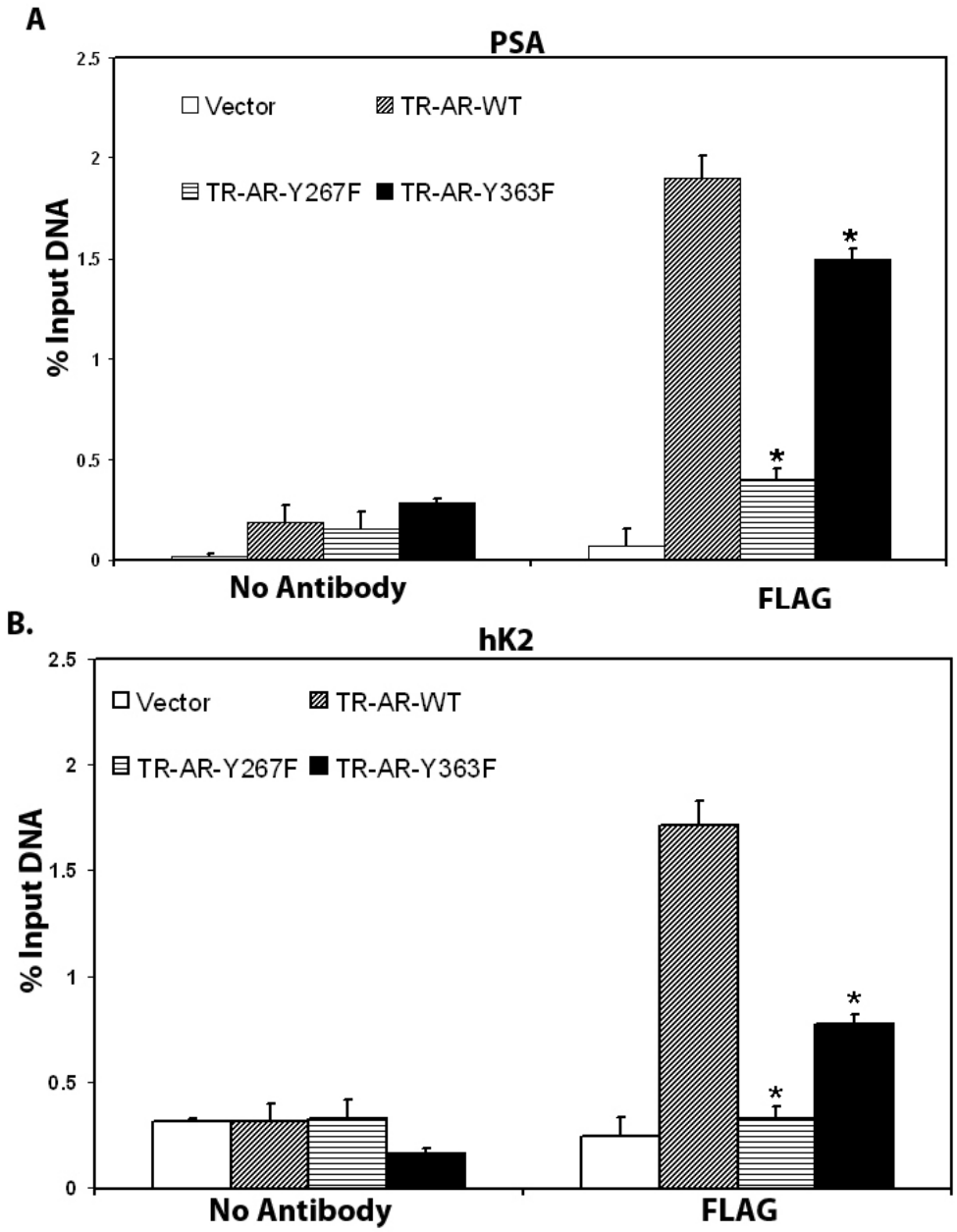


**Figure 3.11. The AR Tyr-267 site has a critical role in truncated AR recruitment to androgen target gene enhancers.** LNCaP cells over expressing TR-WT-AR, TR-AR-Y267F, and TR-AR-Y363F were grown in phenol red free media supplemented with charcoal dextran stripped FBS and serum starved for over night. ChIP analysis for binding of ectopically expressed FLAG-tagged truncated AR protein to the androgen responsive enhancer region of (A) PSA and (B) HK2 were performed using quantitative PCR. Asterisk indicates statistical difference relative to FL-WT-AR ($P < 0.05$) in (A), and TR-WT-AR ($P < 0.05$) in (B), respectively.

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# CHAPTER IV

## CONCLUSIONS & FUTURE DIRECTIONS

## 4.1 Conclusions and future directions

We explored the role of Ack1 target phosphorylation sites (Tyr-267 and Tyr-363) on AR transcriptional activity, cell proliferation, anchorage independent growth and nuclear localization. We showed that mutating Ack1 target phosphorylation sites from tyrosine to phenylalanine (Y267F) inhibited AR transcriptional activity, cell proliferation, anchorage independent growth, and nuclear localization of AR. By studying the affect of these phosphorylation sites in both full- and truncated-AR contexts allowed us to elucidate the role of these phosphorylation sites for both androgen-dependent and -independent activity of AR.

There is emerging evidence from the recent reports that ligand-independent activation of AR by growth factors and phosphorylation mediated AR activity is instrumental for hormone refractory prostate cancer cells to adapt to the selection pressure from ADT. Our work showed that these phosphorylation sites (Tyr-267 and Tyr-363) where Ack1 signals through AR in response to growth factors has a critical role in the transcriptional activity, cell proliferation and anchorage independent growth of LNCaP cells overexpressing truncated AR missing LBD and constitutively active. Recent work by (Guo et al., 2009) showed that the expression of splice variant form of AR missing LBD was elevated in HRPC tumors and they are resistant to therapies targeting LBD of AR. Therefore, future therapeutic modalities targeting NTD of AR as well as these kinases (Ack1 or Src) that signal through these phosphorylation sites in NTD may prevent AR driven HRPC progression and development.

Our study implicated a role for Tyr-267 phosphorylation in transcriptional activity of TR-WT-AR. Furthermore, TR-AR-Y267F overexpressing cells showed reduced cell proliferation, anchorage independent growth compared to that of TR-WT-AR suggesting that AR phosphorylation had a role in activity of truncated AR missing LBD.

The effective prostate cancer treatment in the hormone refractory stage was limited by the shortcomings of current therapeutic modalities that can be improved significantly by understanding the underlying mechanisms of evasion of ADT induced selective pressure. Our report showed that tyrosine phosphorylation sites are essential for both growth factor induced and alternatively spliced truncated AR activity when these constructs were stably overexpressed in LNCaP cells. The exact mechanism of how and when the alternative spliced AR occurs in prostate cancer cells under ADT remains unclear, yet its role in driving the resistance of prostate cancer to therapies and subsequent worsening of patient survival is well known. Therefore, future therapies should include agents that target Ack1 and NTD of AR that is the predominantly transcriptionally active domain of AR that has a critical role in splice variant AR. In fact recent work by Mahajan et al. (2010) showed that Ack1 kinase inhibitor AIM-100 inhibited both Ack1 Tyr-284 and AR Tyr-267 phosphorylation. The inhibition of Tyr-267 phosphorylation declined drastically the ability of AR recruitments to AR target genes (PSA, TMPRSS2, and NKX3.1). Furthermore, AIM-100 induced cell cycle arrest in G0/G1 phase in prostate cancer cells. This initial work implicated that targeting Ack1 in HRPC may become a therapeutic choice in the future.

One of the possible mechanisms whereby AR-Y267F mutant showed defective phenotype might be an aberrant interaction of Tyr-267 mutant AR with chaperone proteins. It has been known that chaperone proteins are involved in critical processes that affect protein folding, activation, mobility, and transcriptional activity of many proteins including AR. Without ligand, AR is located predominantly in the cytoplasm, kept in an inactive conformation by a large complex of chaperone proteins including heat shock proteins (HSPs) HSP-90, -70, and -56 (Elbi et al., 2004; Smith and Toft, 2008). HSPs are responsible for

holding AR at a conformation to bind androgens and its interaction with proteins that mediates nucleocytoplasmic shuttling (Pratt and Toft, 1997; Smith and Toft, 2008). Upon ligand binding, there is a conformational change in the AR that leads to dissociation of the AR-chaperone complex. Next, the AR translocates into the nucleus, interacts with coregulators (coactivator or corepressor), dimerizes, and binds to the AREs present in the promoter or enhancer regions of AR target genes to regulate their transcriptional activity (Edwards and Bartlett, 2005). The ligand induced disassociation of AR from chaperone complex (HSP-100, -90, -70, -60, and -27) is considered as one of regulatory mechanisms of AR signaling (Edwards and Bartlett, 2005). For example, HSP-90 inhibitor, geldanamycin, inhibited the transcriptional activity of AR regulated genes by decreasing the stability and increasing the proteasomal degradation of AR (Vanaja et al., 2002). There are recent reports indicated that elevated co-chaperone activities were detected in hormone refractory prostate cancer (Shatkina et al., 2003; Yamanaka et al., 2005). The elevated expression of Bag-1L was detected in HRPC (Yamanaka et al., 2005) and further study suggested that both $NH_2$ and COOH terminal domain interaction of Bag-1L with the respective domains of AR is an essential requirement for inducing the transcriptional activity of AR (Shatkina et al., 2003). Zoubeidi et al. reported that HSP-27, ATP independent co-chaperone, was phosphorylated (Ser-78 and Ser-82) by ligand induced AR through p38-kinase dependent manner (Zoubeidi et al., 2007). Subsequently, there is an increased AR protein stability, shuttling, and transcriptional activity which led to enhanced prostate cancer cell survival. Interestingly, ligand activation of AR enhanced the presence of HSP-27 in the expense of HSP-90 in the chaperone complex. Both AR and HSP27 were co-localized into the nucleus. The inhibition of the phosphorylation of HSP27 or knocking down HSP-27 with antisense drug OGX-427

enhanced the interaction of AR and HSP-90, increased proteasomal degradation of AR, and concurrently diminished the transcriptional activity of AR. Based on what we know about the chaperone proteins, it would be helpful to investigate the interaction of AR-WT and AR mutants with HSP-90 and HSP-27 co-chaperones to elucidate whether these chaperone proteins have any role in the defective phenotype observed with AR-Y267F mutant AR. Furthermore, future experiments may determine whether AR phosphorylation enhances the stability of AR protein.

The sustained AR activity from the onset of prostate cancer to HRPC led researchers to investigate co-regulatory proteins that interact with AR and affect its transcriptional activity in a cellular context-dependent manner (Heinlein and Chang, 2002). Currently, there are more than 200 co-regulators which have diverse functions depending on the target gene and cellular context (Agoulnik and Weigel, 2009; Heemers and Tindall, 2007). They may enhance (as co-activators) or reduce (as co-repressors) the target gene activity. There are several reports that studied the changes in the expression of AR co-regulators in prostate cancer. Gregory et al. (2001) studied the expression of three nuclear receptor co-activators, transcriptional intermediatory factor 2 (TIF2), steroid receptor co-activator 1 (SRC1), and amplified in breast cancer 1 (AIB1) in samples from benign prostate hyperplasia (BPH), androgen dependent prostate cancer, and androgen independent prostate cancer. They showed that elevated expression of TIF2 and SRC1 correlated with increased AR expression in androgen independent prostate cancer. Co-transfection of AR wild type or mutant AR with TIF2 made AR responsive to non-androgen ligands such as androstenedione, estradiol, and progesterone. This study suggested that overexpression of co-activators may broaden the ligand specificity of AR like AR mutations and may sensitize AR to low levels of androgens.

We have not investigated the expression of co-activators in LNCaP cells over expressing full- or truncated- WT AR or mutant AR. One future experiment may be to determine the expression levels of selected co-activators and co-repressors in the full- or truncated- WT AR overexpressing cells and how mutating Tyr-267 and Tyr-363 sites affect the expression of the co-activators and co-repressors. There are co-activators (such as p160 co-activators) and co-repressor that interact with NTD of AR (Alen et al., 1999; Ueda et al., 2002) Understanding the mechanism of interaction of co-regulators with these phosphorylation sites may help to identify co-regulators involved in survival and proliferation of PCa.

AR phosphorylation by Ack1 and Src in hormone refractory prostate cancer suggested that AR phosphorylation may have a critical role in driving prostate cancer to recurrence that becomes an incurable disease. The result of our studies suggested that growth factor induced AR phosphorylation through Ack1 or Src tyrosine kinases was inhibited by dasatinib, a Src family kinase inhibitor except AR Tyr-267 phosphorylation induced by EGF. Therefore, there may be additional kinases that phosphorylate AR at Tyr-267 in response to EGF treatment of cells. It is possible to determine whether additional kinases become activated by EGF and phosphorylate AR by using proteomic analysis approaches. Identification of other kinases that signal downstream of EGF will increase the number of drug targets that may enhance the efficacy of current prostate cancer therapies.

Our studies concentrated on Ack1 target phosphorylation sites, yet AR is phosphorylated at many sites serine-16, 81, 256, 308, 424, and 650 (Gioeli et al., 2002; Zhou et al., 1995). One future experiment may be to determine the changes in the pattern of phosphorylation of AR at serine/threonine sites in comparison of AR-WT with AR-Y267F or AR-Y363F.

Herein, we specifically studied the effect of AR tyrosine phosphorylation on androgen-independent activation of AR to understand the mechanism of AR activation in HRPC that arose under ADT. However, there are other post-translational modifications such as ubiquitination, SUMOylation can also modulate AR activity (Cardozo and Pagano, 2004; Cheng et al., 2006; Ravid and Hochstrasser, 2008; Wu and Mo, 2007). One future experiment may be to study how AR tyrosine phosphorylation (Tyr-267 or Tyr-363) affects AR ubiquitination and SUMOylation. Characterizing these diverse post-translational modifications of AR may help us understand how AR functions are affected by these modifications. These modifications are tightly regulated and components of dynamic regulation of AR transcriptional machinery. Deregulation of these modifications may play a role in the pathogenesis and progression of PCa. Therefore, understanding the mechanism of action of these modifications such as tyrosine phosphorylation of AR may help develop novel therapeutic approaches to enhance the efficacy of standard therapies.

Three independent groups reported the presence of splice variant AR species missing LBD and constitutively active in castration recurrent cell line models and PCa tissues (Dehm et al., 2008; Guo et al., 2009; Lin et al., 2009). Two groups developed antibodies that specifically recognize truncated AR, but not full length AR to detect truncated AR species in PCa tissues. (Guo et al., 2009; Lin et al., 2009). Their studies showed that although the expression level of truncated AR was lower than that of full length AR, they were able to detect splice variant truncated AR proteins in human PCa tissues. Furthermore, microarray gene expression analysis showed that different groups of genes regulated by full length AR compared to the truncated AR variants suggested that these variants may modulate different biological processes with a selected group of genes. Again, the difference in the mode of

transcription might be due to the set of co-regulators recruited to the AREs by these two different biological entities (full- and truncated-AR). One experiment that might help us understand the difference in the mechanism of action of these two AR forms would be chromatin immunoprecipitation followed by sequencing (Chip-Seq) by using antibodies specifically recognizing full length or truncated AR.

One of the possible clinical implication of having splice variant specific antibody is to determine the expression level of TR-WT-AR during progression of PCa from hormone dependent PCa to HRPC using truncated AR as a biomarker for hormone recurrence. The selective pressure of ADT induces constitutively active splice variants in HRPC cells that evade the therapy. Understanding the mechanism of splice variant AR may help us develop therapeutic approaches that target NTD of AR where predominant AF-1 transcriptional unit resides.

In summary, our work underscores the significance of AR phosphorylation in AR transcriptional activity, cell proliferation, anchorage independent growth, nuclear localization and DNA binding under androgen depleted conditions. Having phospho-Tyr-267 and Tyr-534 AR antibodies as well as Ack1 phopho-Tyr-284 may allow us to measure the effectiveness of therapeutic targeting of Src and Ack1 in HRPC. In the future, identification of additional kinases signaling through Tyr-267 will enhance our understanding of the role of these AR phosphorylation sites in HRPC and may help in developing therapeutic approaches that target these kinases.

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# CHAPTER V

APPENDIX

**Table 5.1 The list of the genes that are significantly up regulated in FL-WT-AR compared to vector control**

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P145694 | ASNS ^ Asparagine synthetase ^ | 1.78 |
| AGI_HUM1_OLIGO_A_23_P117782 | FLJ11196 ^ Acheron ^ | 3.51 |
| AGI_HUM1_OLIGO_A_24_P941359 | C6orf32 ^ Chromosome 6 open reading frame 32 ^ | 14.39 |
| AGI_HUM1_OLIGO_A_23_P120845 | XBP1 ^ X-box binding protein 1 ^ | 1.71 |
| AGI_HUM1_OLIGO_A_23_P314584 | MAPKAPK3 ^ Mitogen-activated protein kinase-activated protein kinase 3 ^ | 1.44 |
| AGI_HUM1_OLIGO_A_24_P250333 | SNRPA ^ Small nuclear ribonucleoprotein polypeptide A ^ | 1.23 |
| AGI_HUM1_OLIGO_A_32_P46594 | LOC145837 ^ Hypothetical protein LOC145837 ^ | 1.62 |
| AGI_HUM1_OLIGO_A_23_P17855 | HRIHFB2122 ^ Tara-like protein ^ | 2.35 |
| AGI_HUM1_OLIGO_A_32_P51518 | ^ CDNA FLJ40901 fis, clone UTERU2003704 ^ | 2.40 |
| AGI_HUM1_OLIGO_A_24_P107695 | ACTN1 ^ Actinin, alpha 1 ^ | 1.55 |
| AGI_HUM1_OLIGO_A_23_P305981 | LOC388152 ^ LOC388161 ^ | 2.05 |
| AGI_HUM1_OLIGO_A_23_P1615 | FIBP ^ Fibroblast growth factor (acidic) intracellular binding protein ^ | 1.30 |
| AGI_HUM1_OLIGO_A_23_P394014 | M-RIP ^ Myosin phosphatase-Rho interacting protein ^ | 1.59 |
| AGI_HUM1_OLIGO_A_23_P1682 | LOC120224 ^ Hypothetical protein BC016153 ^ | 11.60 |
| AGI_HUM1_OLIGO_A_24_P45005 | NPEPL1 ^ Aminopeptidase-like 1 ^ | 1.42 |
| AGI_HUM1_OLIGO_A_23_P312646 | LOC286257 ^ Hypothetical protein LOC286257 ^ | 1.44 |
| AGI_HUM1_OLIGO_A_24_P256552 | CSTF3 ^ Hypothetical protein LOC283267 ^ | 1.46 |
| AGI_HUM1_OLIGO_A_24_P204144 | Uncharacterized gene | 1.69 |
| AGI_HUM1_OLIGO_A_24_P109661 | Uncharacterized gene | 1.26 |
| AGI_HUM1_OLIGO_A_23_P132285 | Uncharacterized gene | 1.48 |
| AGI_HUM1_OLIGO_A_32_P2730 | Uncharacterized gene | 3.43 |
| AGI_HUM1_OLIGO_A_23_P63897 | CTBP2 ^ C-terminal binding protein 2 ^ | 1.23 |
| AGI_HUM1_OLIGO_A_24_P350124 | KIAA1618 ^ KIAA1618 ^ | 1.66 |
| | | |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P254944 | GSTT1 ^ Glutathione S-transferase theta 1 ^ | 1.73 |
| AGI_HUM1_OLIGO_A_24_P595460 | NUCB2 ^ Nucleobindin 2 ^ | 2.32 |
| AGI_HUM1_OLIGO_A_23_P379550 | YARS ^ Tyrosyl-tRNA synthetase ^ | 2.07 |
| AGI_HUM1_OLIGO_A_23_P155009 | C22orf3 ^ Chromosome 22 open reading frame 3 ^ | 1.49 |
| AGI_HUM1_OLIGO_A_23_P117992 | KIAA0703 ^ KIAA0703 gene product ^ | 2.61 |
| AGI_HUM1_OLIGO_A_24_P814246 | Uncharacterized gene | 1.79 |
| AGI_HUM1_OLIGO_A_32_P217709 | RAC1 ^ Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1) ^ | 1.38 |
| AGI_HUM1_OLIGO_A_24_P95439 | Uncharacterized gene | 2.17 |
| AGI_HUM1_OLIGO_A_24_P827738 | Uncharacterized gene | 2.17 |
| AGI_HUM1_OLIGO_A_23_P119943 | IGFBP2 ^ Insulin-like growth factor binding protein 2, 36kDa ^ | 1.38 |
| AGI_HUM1_OLIGO_A_23_P54758 | MIR16 ^ Membrane interacting protein of RGS16 ^ | 1.55 |
| AGI_HUM1_OLIGO_A_24_P306558 | KIAA1049 ^ KIAA1049 protein ^ | 2.06 |
| AGI_HUM1_OLIGO_A_32_P135348 | Uncharacterized gene | 1.94 |
| AGI_HUM1_OLIGO_A_24_P936767 | TRIM25 ^ Tripartite motif-containing 25 ^ | 1.79 |
| AGI_HUM1_OLIGO_A_32_P184796 | RPLP0 ^ Ribosomal protein, large, P0 ^ | 1.67 |
| AGI_HUM1_OLIGO_A_23_P9472 | VPS13A ^ Vacuolar protein sorting 13A (yeast) ^ | 1.84 |
| AGI_HUM1_OLIGO_A_23_P9688 | DNCLI2 ^ Dynein, cytoplasmic, light intermediate polypeptide 2 ^ | 1.90 |
| AGI_HUM1_OLIGO_A_23_P208030 | SYT4 ^ Synaptotagmin IV ^ | 2.25 |
| AGI_HUM1_OLIGO_A_32_P21255 | SLC30A4 ^ Solute carrier family 30 (zinc transporter), member 4 ^ | 3.05 |
| AGI_HUM1_OLIGO_A_32_P194072 | DKFZP434B0335 ^ DKFZP434B0335 protein ^ | 2.05 |
| AGI_HUM1_OLIGO_A_23_P155027 | ZCWCC1 ^ Zinc finger, CW type with coiled-coil domain 1 ^ | 1.13 |
| AGI_HUM1_OLIGO_A_24_P212152 | FLJ14346 ^ Hypothetical protein FLJ14346 ^ | 1.27 |
| | | |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P53541 | CHD4 ^ Chromodomain helicase DNA binding protein 4 ^ | 2.02 |
| AGI_HUM1_OLIGO_A_24_P398432 | CENTD2 ^ Centaurin, delta 2 ^ | 1.61 |
| AGI_HUM1_OLIGO_A_23_P253029 | BOK ^ BCL2-related ovarian killer ^ | 1.94 |
| AGI_HUM1_OLIGO_A_23_P390528 | DUSP8 ^ Dual specificity phosphatase 8 ^ | 1.88 |
| AGI_HUM1_OLIGO_A_23_P204472 | RPLP0 ^ Ribosomal protein, large, P0 ^ | 2.07 |
| AGI_HUM1_OLIGO_A_23_P101707 | M6PRBP1 ^ Mannose-6-phosphate receptor binding protein 1 ^ | 1.46 |
| AGI_HUM1_OLIGO_A_24_P412238 | MUS81 ^ MUS81 endonuclease homolog (yeast) ^ | 1.33 |
| AGI_HUM1_OLIGO_A_24_P152635 | TMX2 ^ Thioredoxin-related transmembrane protein 2 ^ | 1.33 |
| AGI_HUM1_OLIGO_A_23_P52569 | NUDT8 ^ Nudix (nucleoside diphosphate linked moiety X)-type motif 8 ^ | 1.81 |
| AGI_HUM1_OLIGO_A_23_P60683 | STK24 ^ Serine/threonine kinase 24 (STE20 homolog, yeast) ^ | 1.44 |
| AGI_HUM1_OLIGO_A_23_P216689 | BRD3 ^ Bromodomain containing 3 ^ | 2.32 |
| AGI_HUM1_OLIGO_A_24_P938135 | Uncharacterized gene | 1.87 |
| AGI_HUM1_OLIGO_A_23_P320478 | FLJ22795 ^ Similar to golgi autoantigen golgin subfamily a2-like ^ | 2.04 |
| AGI_HUM1_OLIGO_A_24_P50554 | Uncharacterized gene | 1.08 |
| AGI_HUM1_OLIGO_A_24_P100234 | ZCWCC1 ^ Zinc finger, CW type with coiled-coil domain 1 ^ | 1.51 |
| AGI_HUM1_OLIGO_A_24_P490857 | ^ CDNA clone IMAGE:5263531, partial cds ^ | 1.54 |
| AGI_HUM1_OLIGO_A_24_P592570 | Uncharacterized gene | 1.47 |
| AGI_HUM1_OLIGO_A_23_P213102 | KIAA0992 ^ Palladin ^ | 1.48 |
| AGI_HUM1_OLIGO_A_24_P144936 | PKD1-like ^ Polycystic kidney disease 1-like ^ | 2.11 |
| AGI_HUM1_OLIGO_A_23_P113405 | CNNM3 ^ Cyclin M3 ^ | 1.65 |
| AGI_HUM1_OLIGO_A_23_P118353 | SRP68 ^ Signal recognition particle 68kDa ^ | 1.59 |
| AGI_HUM1_OLIGO_A_24_P247233 | Uncharacterized gene | 1.27 |
| | | |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P87763 | EEF2 ^ Eukaryotic translation elongation factor 2 ^ | 2.11 |
| AGI_HUM1_OLIGO_A_23_P128246 | HYPE ^ Huntingtin interacting protein E ^ | 1.92 |
| AGI_HUM1_OLIGO_A_32_P206899 | Uncharacterized gene | 2.39 |
| AGI_HUM1_OLIGO_A_23_P401106 | PDE2A ^ Phosphodiesterase 2A, cGMP-stimulated ^ | 2.85 |
| AGI_HUM1_OLIGO_A_23_P52939 | SLC43A1 ^ Solute carrier family 43, member 1 ^ | 2.37 |
| AGI_HUM1_OLIGO_A_23_P129221 | FAH ^ Fumarylacetoacetate hydrolase (fumarylacetoacetase) ^ | 1.59 |
| AGI_HUM1_OLIGO_A_24_P6083 | C22orf16 ^ Chromosome 22 open reading frame 16 ^ | 1.86 |
| AGI_HUM1_OLIGO_A_23_P48747 | DHRS1 ^ Dehydrogenase/reductase (SDR family) member 1 ^ | 1.61 |
| AGI_HUM1_OLIGO_A_23_P341065 | SELO ^ Selenoprotein O ^ | 1.77 |
| AGI_HUM1_OLIGO_A_23_P334021 | IGF2R ^ Insulin-like growth factor 2 receptor ^ | 1.78 |
| AGI_HUM1_OLIGO_A_23_P120488 | NPEPL1 ^ Aminopeptidase-like 1 ^ | 1.43 |
| AGI_HUM1_OLIGO_A_23_P101013 | EVER1 ^ Epidermodysplasia verruciformis 1 ^ | 2.00 |
| AGI_HUM1_OLIGO_A_23_P217015 | SET ^ SET translocation (myeloid leukemia-associated) ^ | 1.82 |
| AGI_HUM1_OLIGO_A_24_P216087 | PD2 ^ Hypothetical protein F23149_1 ^ | 1.32 |
| AGI_HUM1_OLIGO_A_24_P188460 | Uncharacterized gene | 1.58 |
| AGI_HUM1_OLIGO_A_32_P49334 | LOC284889 ^ Hypothetical protein LOC284889 ^ | 1.55 |
| AGI_HUM1_OLIGO_A_24_P803885 | LOC149134 ^ Hypothetical protein LOC149134 ^ | 1.69 |
| AGI_HUM1_OLIGO_A_32_P159820 | Uncharacterized gene | 1.25 |
| AGI_HUM1_OLIGO_A_23_P80377 | PACSIN2 ^ Protein kinase C and casein kinase substrate in neurons 2 ^ | 2.11 |
| AGI_HUM1_OLIGO_A_23_P407628 | MYST3 ^ MYST histone acetyltransferase (monocytic leukemia) 3 ^ | 1.39 |
| AGI_HUM1_OLIGO_A_23_P368028 | TP53I11 ^ Tumor protein p53 inducible protein 11 ^ | 1.58 |
| AGI_HUM1_OLIGO_A_23_P24723 | HSPC196 ^ Hypothetical protein HSPC196 ^ | 1.55 |
| AGI_HUM1_OLIGO_A_23_P40108 | COL9A3 ^ Collagen, type IX, alpha 3 ^ | 1.32 |
| | | |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P76690 | UCHL3 ^ Ubiquitin carboxyl-terminal esterase L3 (ubiquitin thiolesterase) ^ | 1.42 |
| AGI_HUM1_OLIGO_A_32_P36136 | Uncharacterized gene | 1.50 |
| AGI_HUM1_OLIGO_A_24_P281374 | Uncharacterized gene | 1.18 |
| AGI_HUM1_OLIGO_A_24_P136484 | MGC15875 ^ Hypothetical protein MGC15875 ^ | 1.65 |
| AGI_HUM1_OLIGO_A_23_P109606 | SBF1 ^ SET binding factor 1 ^ | 2.27 |
| AGI_HUM1_OLIGO_A_23_P58622 | UBE2D2 ^ Ubiquitin-conjugating enzyme E2D 2 (UBC4/5 homolog, yeast) ^ | 1.35 |
| AGI_HUM1_OLIGO_A_24_P127828 | VCP ^ Valosin-containing protein ^ | 1.64 |
| AGI_HUM1_OLIGO_A_23_P35848 | NADSYN1 ^ NAD synthetase 1 ^ | 1.38 |
| AGI_HUM1_OLIGO_A_23_P388780 | DMAP1 ^ DNA methyltransferase 1 associated protein 1 ^ | 1.26 |
| AGI_HUM1_OLIGO_A_24_P99071 | RANBP5 ^ RAN binding protein 5 ^ | 1.75 |
| AGI_HUM1_OLIGO_A_23_P162486 | PTPN6 ^ Protein tyrosine phosphatase, non-receptor type 6 ^ | 1.75 |
| AGI_HUM1_OLIGO_A_23_P159539 | ASMTL ^ Acetylserotonin O-methyltransferase-like ^ | 1.66 |
| AGI_HUM1_OLIGO_A_23_P103120 | GTPBP1 ^ GTP binding protein 1 ^ | 1.48 |
| AGI_HUM1_OLIGO_A_23_P169629 | SHMT2 ^ Serine hydroxymethyltransferase 2 (mitochondrial) ^ | 1.52 |
| AGI_HUM1_OLIGO_A_24_P205364 | SHMT1 ^ Serine hydroxymethyltransferase 1 (soluble) ^ | 1.19 |
| AGI_HUM1_OLIGO_A_23_P16538 | ELL ^ Elongation factor RNA polymerase II ^ | 1.24 |
| AGI_HUM1_OLIGO_A_23_P132675 | UQCRC1 ^ Ubiquinol-cytochrome c reductase core protein I ^ | 1.40 |
| AGI_HUM1_OLIGO_A_24_P944751 | Uncharacterized gene | 2.41 |
| AGI_HUM1_OLIGO_A_23_P205929 | DPP8 ^ Dipeptidylpeptidase 8 ^ | 1.39 |
| AGI_HUM1_OLIGO_A_24_P100228 | XBP1 ^ X-box binding protein 1 ^ | 2.33 |
| AGI_HUM1_OLIGO_A_23_P167559 | FLJ31951 ^ Hypothetical protein FLJ31951 ^ | 1.53 |
| | | |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P196400 | PHIP ^ Pleckstrin homology domain interacting protein ^ | 1.36 |
| AGI_HUM1_OLIGO_A_23_P140170 | SEC23A ^ Sec23 homolog A (S. cerevisiae) ^ | 1.33 |
| AGI_HUM1_OLIGO_A_23_P355385 | PPP6C ^ Protein phosphatase 6, catalytic subunit ^ | 1.26 |
| AGI_HUM1_OLIGO_A_24_P344510 | KLK3 ^ Kallikrein 3, (prostate specific antigen) ^ | 4.63 |
| AGI_HUM1_OLIGO_A_32_P146898 | Uncharacterized gene | 1.78 |
| AGI_HUM1_OLIGO_A_24_P213161 | NALP2 ^ NACHT, leucine rich repeat and PYD containing 2 ^ | 1.66 |
| AGI_HUM1_OLIGO_A_24_P267293 | SARM1 ^ Sterile alpha and TIR motif containing 1 ^ | 1.62 |
| AGI_HUM1_OLIGO_A_24_P97785 | PURA ^ Purine-rich element binding protein A ^ | 1.53 |
| AGI_HUM1_OLIGO_A_24_P98249 | TACC1 ^ Transforming, acidic coiled-coil containing protein 1 ^ | 2.04 |
| AGI_HUM1_OLIGO_A_23_P88046 | FLJ10769 ^ Hypothetical protein FLJ10769 ^ | 1.72 |
| AGI_HUM1_OLIGO_A_23_P375147 | Uncharacterized gene | 1.55 |
| AGI_HUM1_OLIGO_A_23_P322845 | HTPAP ^ HTPAP protein ^ | 1.44 |
| AGI_HUM1_OLIGO_A_23_P128073 | SMARCC2 ^ SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2 ^ | 1.74 |
| AGI_HUM1_OLIGO_A_32_P114246 | USP47 ^ Ubiquitin specific protease 47 ^ | 1.54 |
| AGI_HUM1_OLIGO_A_23_P141032 | COX4I1 ^ Cytochrome c oxidase subunit IV isoform 1 ^ | 1.39 |
| AGI_HUM1_OLIGO_A_24_P115971 | RIC-8 ^ Likely ortholog of mouse synembryn ^ | 1.42 |
| AGI_HUM1_OLIGO_A_23_P121795 | ARGBP2 ^ Arg/Abl-interacting protein ArgBP2 ^ | 1.89 |
| AGI_HUM1_OLIGO_A_24_P940576 | KIAA1340 ^ KIAA1340 protein ^ | 1.86 |
| AGI_HUM1_OLIGO_A_23_P129738 | LLGL2 ^ Lethal giant larvae homolog 2 (Drosophila) ^ | 1.44 |
| AGI_HUM1_OLIGO_A_24_P305467 | p66alpha ^ P66 alpha ^ | 1.42 |
| AGI_HUM1_OLIGO_A_23_P26713 | RPL23 ^ Ribosomal protein L23 ^ | 1.18 |
| AGI_HUM1_OLIGO_A_23_P115167 | TA-LRRP ^ T-cell activation leucine repeat-rich protein ^ | 1.46 |
| AGI_HUM1_OLIGO_A_23_P395703 | HBXAP ^ Hepatitis B virus x associated protein ^ | 1.48 |
| | | |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P149766 | MGC10765 ^ Hypothetical protein MGC10765 ^ | 1.59 |
| AGI_HUM1_OLIGO_A_23_P253177 | STK24 ^ Serine/threonine kinase 24 (STE20 homolog, yeast) ^ | 1.71 |
| AGI_HUM1_OLIGO_A_23_P124912 | NPEPPS ^ Aminopeptidase puromycin sensitive ^ | 1.17 |
| AGI_HUM1_OLIGO_A_24_P324405 | ANKRD11 ^ Ankyrin repeat domain 11 ^ | 2.30 |
| AGI_HUM1_OLIGO_A_23_P115534 | PKD1-like ^ Polycystic kidney disease 1-like ^ | 1.79 |
| AGI_HUM1_OLIGO_A_23_P372771 | C15orf17 ^ Chromosome 15 open reading frame 17 ^ | 1.88 |
| AGI_HUM1_OLIGO_A_23_P12849 | FBXO18 ^ F-box protein, helicase, 18 ^ | 1.38 |
| AGI_HUM1_OLIGO_A_23_P64938 | C3F ^ Putative protein similar to nessy (Drosophila) ^ | 1.93 |
| AGI_HUM1_OLIGO_A_23_P130886 | FLJ14981 ^ Hypothetical protein FLJ14981 ^ | 1.37 |
| AGI_HUM1_OLIGO_A_32_P104063 | ^ Hypothetical gene supported by AF275804 ^ | 1.58 |
| AGI_HUM1_OLIGO_A_23_P395172 | ABHD2 ^ Abhydrolase domain containing 2 ^ | 2.19 |
| AGI_HUM1_OLIGO_A_23_P155335 | PLD1 ^ Phospholipase D1, phophatidylcholine-specific ^ | 2.36 |
| AGI_HUM1_OLIGO_A_24_P231494 | DNPEP ^ Aspartyl aminopeptidase ^ | 1.33 |
| AGI_HUM1_OLIGO_A_32_P89371 | RANBP2L1 ^ RAN binding protein 2-like 1 ^ | 2.06 |
| AGI_HUM1_OLIGO_A_23_P162970 | IPO4 ^ Importin 4 ^ | 1.50 |

**Table 5.2 The list of the genes that are significantly down regulated in FL-WT-AR compared to vector control.**

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P254271 | TUBB6 ^ Tubulin, beta 6 ^ | 0.11 |
| AGI_HUM1_OLIGO_A_23_P252704 | LOC51123 ^ HSPC038 protein ^ | 0.52 |
| AGI_HUM1_OLIGO_A_24_P83968 | Uncharacterized gene | 0.84 |
| AGI_HUM1_OLIGO_A_23_P51508 | DUSP12 ^ Dual specificity phosphatase 12 ^ | 0.70 |
| AGI_HUM1_OLIGO_A_24_P88850 | MRAS ^ Muscle RAS oncogene homolog ^ | 0.61 |
| AGI_HUM1_OLIGO_A_23_P309361 | FLJ30525 ^ Hypothetical protein FLJ30525 ^ | 0.03 |
| AGI_HUM1_OLIGO_A_23_P74981 | MGC12466 ^ Hypothetical protein MGC12466 ^ | 0.42 |
| AGI_HUM1_OLIGO_A_23_P201996 | WAC ^ WW domain containing adaptor with coiled-coil ^ | 0.79 |
| AGI_HUM1_OLIGO_A_24_P327886 | TCEA3 ^ Transcription elongation factor A (SII), 3 ^ | 0.13 |
| AGI_HUM1_OLIGO_A_24_P143189 | TMSL3 ^ Thymosin-like 3 ^ | 0.21 |
| AGI_HUM1_OLIGO_A_23_P141044 | LOC146542 ^ Similar to hypothetical protein MGC13138 ^ | 0.47 |
| AGI_HUM1_OLIGO_A_23_P200901 | ENSA ^ Endosulfine alpha ^ | 0.72 |
| AGI_HUM1_OLIGO_A_24_P384990 | ^ Homo sapiens, clone IMAGE:4157625, mRNA ^ | 0.46 |
| AGI_HUM1_OLIGO_A_24_P374516 | TMSB4X ^ Thymosin, beta 4, X-linked ^ | 0.26 |
| AGI_HUM1_OLIGO_A_23_P252711 | LACTB2 ^ Lactamase, beta 2 ^ | 0.57 |
| AGI_HUM1_OLIGO_A_23_P201731 | TRAF5 ^ TNF receptor-associated factor 5 ^ | 0.14 |
| AGI_HUM1_OLIGO_A_23_P200216 | MAGOH ^ Mago-nashi homolog, proliferation-associated (Drosophila) ^ | 0.43 |
| AGI_HUM1_OLIGO_A_23_P412577 | ANKRD29 ^ Ankyrin repeat domain 29 ^ | 0.17 |
| AGI_HUM1_OLIGO_A_23_P385034 | E2F3 ^ E2F transcription factor 3 ^ | 0.57 |
| AGI_HUM1_OLIGO_A_23_P256735 | PGCP ^ Plasma glutamate carboxypeptidase ^ | 0.29 |
| AGI_HUM1_OLIGO_A_24_P201064 | PPP1R3B ^ Protein phosphatase 1, regulatory (inhibitor) subunit 3B ^ | 0.49 |
| AGI_HUM1_OLIGO_A_23_P414771 | Uncharacterized gene | 0.64 |
| | | |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P85726 | MGC9084 ^ Hypothetical protein MGC9084 ^ | 0.58 |
| AGI_HUM1_OLIGO_A_23_P49546 | GRIN2C ^ Glutamate receptor, ionotropic, N-methyl D-aspartate 2C ^ | 0.23 |
| AGI_HUM1_OLIGO_A_23_P208090 | MBD1 ^ Methyl-CpG binding domain protein 1 ^ | 0.66 |
| AGI_HUM1_OLIGO_A_23_P103905 | Ufc1 ^ Ufm1-conjugating enzyme 1 ^ | 0.56 |
| AGI_HUM1_OLIGO_A_24_P719948 | ^ Full length insert cDNA YQ80D07 ^ | 0.50 |
| AGI_HUM1_OLIGO_A_23_P251002 | Uncharacterized gene | 0.60 |
| AGI_HUM1_OLIGO_A_23_P123672 | TDRD7 ^ Tudor domain containing 7 ^ | 0.56 |
| AGI_HUM1_OLIGO_A_32_P44274 | CHTF18 ^ CTF18, chromosome transmission fidelity factor 18 homolog (S. cerevisiae) ^ | 0.74 |
| AGI_HUM1_OLIGO_A_24_P230416 | Uncharacterized gene | 0.66 |
| AGI_HUM1_OLIGO_A_23_P419239 | ETNK1 ^ Ethanolamine kinase 1 ^ | 0.64 |
| AGI_HUM1_OLIGO_A_23_P52101 | NQO3A2 ^ NAD(P)H:quinone oxidoreductase type 3, polypeptide A2 ^ | 0.73 |
| AGI_HUM1_OLIGO_A_23_P161190 | VIM ^ Vimentin ^ | 0.33 |
| AGI_HUM1_OLIGO_A_24_P209204 | C6orf62 ^ Chromosome 6 open reading frame 62 ^ | 0.55 |
| AGI_HUM1_OLIGO_A_23_P122545 | RDBP ^ RD RNA binding protein ^ | 0.93 |
| AGI_HUM1_OLIGO_A_23_P86570 | ANXA7 ^ Annexin A7 ^ | 0.73 |
| AGI_HUM1_OLIGO_A_32_P41574 | PXN ^ Paxillin ^ | 0.70 |
| AGI_HUM1_OLIGO_A_23_P155857 | NUDT6 ^ Nudix (nucleoside diphosphate linked moiety X)-type motif 6 ^ | 0.41 |
| AGI_HUM1_OLIGO_A_24_P382119 | MTMR4 ^ Myotubularin related protein 4 ^ | 0.84 |
| AGI_HUM1_OLIGO_A_23_P160546 | FLJ11280 ^ Hypothetical protein FLJ11280 ^ | 0.30 |
| AGI_HUM1_OLIGO_A_24_P374513 | Uncharacterized gene | 0.26 |
| AGI_HUM1_OLIGO_A_23_P114662 | CRYZ ^ Crystallin, zeta (quinone reductase) ^ | 0.78 |
| AGI_HUM1_OLIGO_A_23_P127579 | PTS ^ 6-pyruvoyltetrahydropterin synthase ^ | 0.72 |
| AGI_HUM1_OLIGO_A_23_P407583 | PEG3 ^ Paternally expressed 3 ^ | 0.11 |
| | | |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P92765 | Uncharacterized gene | 0.77 |
| AGI_HUM1_OLIGO_A_23_P501624 | UGT2B17 ^ UDP glycosyltransferase 2 family, polypeptide B17 ^ | 0.82 |
| AGI_HUM1_OLIGO_A_23_P85952 | FLJ22457 ^ Hypothetical protein FLJ22457 ^ | 0.13 |
| AGI_HUM1_OLIGO_A_23_P42435 | DTNBP1 ^ Dystrobrevin binding protein 1 ^ | 0.69 |
| AGI_HUM1_OLIGO_A_23_P371824 | TUFT1 ^ Tuftelin 1 ^ | 0.60 |
| AGI_HUM1_OLIGO_A_24_P178663 | Uncharacterized gene | 0.56 |
| AGI_HUM1_OLIGO_A_23_P116235 | Uncharacterized gene | 0.20 |
| AGI_HUM1_OLIGO_A_23_P48676 | PYGL ^ Phosphorylase, glycogen; liver (Hers disease, glycogen storage disease type VI) ^ | 0.23 |
| AGI_HUM1_OLIGO_A_24_P230009 | Uncharacterized gene | 0.63 |
| AGI_HUM1_OLIGO_A_23_P34375 | TCEA3 ^ Transcription elongation factor A (SII), 3 ^ | 0.23 |
| AGI_HUM1_OLIGO_A_23_P110362 | MAP2K1IP1 ^ Mitogen-activated protein kinase kinase 1 interacting protein 1 ^ | 0.58 |
| AGI_HUM1_OLIGO_A_23_P57137 | C20orf29 ^ Chromosome 20 open reading frame 29 ^ | 0.77 |
| AGI_HUM1_OLIGO_A_32_P54018 | KIAA1143 ^ KIAA1143 protein ^ | 0.69 |
| AGI_HUM1_OLIGO_A_23_P160518 | TRIM45 ^ Tripartite motif-containing 45 ^ | 0.61 |
| AGI_HUM1_OLIGO_A_23_P70307 | SMOC2 ^ SPARC related modular calcium binding 2 ^ | 0.29 |
| AGI_HUM1_OLIGO_A_24_P261083 | C8orf1 ^ Chromosome 8 open reading frame 1 ^ | 0.69 |
| AGI_HUM1_OLIGO_A_23_P65000 | HSU79274 ^ Protein predicted by clone 23733 ^ | 0.76 |
| AGI_HUM1_OLIGO_A_23_P141362 | FZD2 ^ Frizzled homolog 2 (Drosophila) ^ | 0.35 |
| AGI_HUM1_OLIGO_A_23_P91350 | Uncharacterized gene | 0.55 |
| AGI_HUM1_OLIGO_A_23_P160567 | ZMYND12 ^ Zinc finger, MYND domain containing 12 ^ | 0.56 |
| AGI_HUM1_OLIGO_A_24_P49539 | MRPL55 ^ Mitochondrial ribosomal protein L55 ^ | 0.75 |
| AGI_HUM1_OLIGO_A_23_P257296 | TFF3 ^ Trefoil factor 3 (intestinal) ^ | 0.51 |
| AGI_HUM1_OLIGO_A_24_P339611 | PDCD5 ^ Programmed cell death 5 ^ | 0.78 |
| | | |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P48771 | C14orf159 ^ Chromosome 14 open reading frame 159 ^ | 0.59 |
| AGI_HUM1_OLIGO_A_23_P92490 | DHRS6 ^ Dehydrogenase/reductase (SDR family) member 6 ^ | 0.42 |
| AGI_HUM1_OLIGO_A_24_P400355 | C7orf20 ^ Chromosome 7 open reading frame 20 ^ | 0.72 |
| AGI_HUM1_OLIGO_A_32_P183356 | Uncharacterized gene | 0.48 |
| AGI_HUM1_OLIGO_A_23_P393425 | PAPD4 ^ PAP associated domain containing 4 ^ | 0.74 |
| AGI_HUM1_OLIGO_A_24_P104174 | TAOK3 ^ TAO kinase 3 ^ | 0.56 |
| AGI_HUM1_OLIGO_A_23_P20814 | DDX58 ^ DEAD (Asp-Glu-Ala-Asp) box polypeptide 58 ^ | 0.43 |
| AGI_HUM1_OLIGO_A_23_P102364 | NGEF ^ Neuronal guanine nucleotide exchange factor ^ | 0.54 |
| AGI_HUM1_OLIGO_A_24_P302172 | PTGFR ^ Prostaglandin F receptor (FP) ^ | 0.20 |
| AGI_HUM1_OLIGO_A_23_P46507 | CGI-143 ^ CGI-143 protein ^ | 0.48 |
| AGI_HUM1_OLIGO_A_32_P233314 | EXOC8 ^ Exocyst complex component 8 ^ | 0.61 |
| AGI_HUM1_OLIGO_A_23_P410017 | MGC10233 ^ Hypothetical protein MGC10233 ^ | 0.53 |
| AGI_HUM1_OLIGO_A_32_P351277 | ^ LOC440330 ^ | 0.34 |
| AGI_HUM1_OLIGO_A_32_P232035 | Uncharacterized gene | 0.85 |
| AGI_HUM1_OLIGO_A_24_P382253 | P15RS ^ Hypothetical protein FLJ10656 ^ | 0.64 |
| AGI_HUM1_OLIGO_A_24_P8220 | HS6ST1 ^ Heparan sulfate 6-O-sulfotransferase 1 ^ | 0.70 |
| AGI_HUM1_OLIGO_A_24_P149124 | C5orf13 ^ Chromosome 5 open reading frame 13 ^ | 0.71 |
| AGI_HUM1_OLIGO_A_23_P81770 | PTP4A1 ^ Protein tyrosine phosphatase type IVA, member 1 ^ | 0.48 |
| AGI_HUM1_OLIGO_A_23_P148345 | RNF128 ^ Ring finger protein 128 ^ | 0.35 |
| AGI_HUM1_OLIGO_A_23_P112481 | AQP3 ^ Aquaporin 3 ^ | 0.39 |
| AGI_HUM1_OLIGO_A_23_P141484 | FLJ10700 ^ Hypothetical protein FLJ10700 ^ | 0.64 |
| AGI_HUM1_OLIGO_A_23_P145408 | FUCA2 ^ Fucosidase, alpha-L- 2, plasma ^ | 0.09 |
| AGI_HUM1_OLIGO_A_23_P77731 | CRYM ^ Crystallin, mu ^ | 0.48 |
| AGI_HUM1_OLIGO_A_23_P391506 | IVNS1ABP ^ Influenza virus NS1A binding protein ^ | 0.46 |
| | | |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P501435 | CSRP2BP ^ CSRP2 binding protein ^ | 0.63 |
| AGI_HUM1_OLIGO_A_23_P19482 | DDAH2 ^ Dimethylarginine dimethylaminohydrolase 2 ^ | 0.57 |
| AGI_HUM1_OLIGO_A_23_P142075 | ACP5 ^ Acid phosphatase 5, tartrate resistant ^ | 0.18 |
| AGI_HUM1_OLIGO_A_24_P366495 | Uncharacterized gene | 0.69 |
| AGI_HUM1_OLIGO_A_24_P928765 | Uncharacterized gene | 0.49 |
| AGI_HUM1_OLIGO_A_23_P432448 | MGC3771 ^ Hypothetical protein MGC3771 ^ | 0.47 |
| AGI_HUM1_OLIGO_A_23_P97328 | C1orf35 ^ Chromosome 1 open reading frame 35 ^ | 0.51 |
| AGI_HUM1_OLIGO_A_23_P89249 | ERBB2 ^ V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian) ^ | 0.75 |
| AGI_HUM1_OLIGO_A_23_P146354 | POMT1 ^ Protein-O-mannosyltransferase 1 ^ | 0.86 |
| AGI_HUM1_OLIGO_A_32_P171061 | ASCL2 ^ Achaete-scute complex-like 2 (Drosophila) ^ | 0.25 |
| AGI_HUM1_OLIGO_A_23_P356554 | BAG2 ^ BCL2-associated athanogene 2 ^ | 0.29 |
| AGI_HUM1_OLIGO_A_23_P87591 | YEATS4 ^ YEATS domain containing 4 ^ | 0.73 |
| AGI_HUM1_OLIGO_A_32_P166921 | TK2 ^ Thymidine kinase 2, mitochondrial ^ | 0.55 |
| AGI_HUM1_OLIGO_A_32_P119830 | PEG3 ^ Paternally expressed 3 ^ | 0.16 |
| AGI_HUM1_OLIGO_A_23_P200930 | MTR ^ 5-methyltetrahydrofolate-homocysteine methyltransferase ^ | 0.61 |
| AGI_HUM1_OLIGO_A_23_P385217 | ARL10C ^ ADP-ribosylation factor-like 10C ^ | 0.81 |
| AGI_HUM1_OLIGO_A_23_P416305 | Uncharacterized gene | 0.53 |
| AGI_HUM1_OLIGO_A_23_P82674 | GBAS ^ Glioblastoma amplified sequence ^ | 0.83 |
| AGI_HUM1_OLIGO_A_23_P62764 | MGC1203 ^ Hypothetical protein MGC1203 ^ | 0.54 |
| AGI_HUM1_OLIGO_A_24_P233995 | FLJ22390 ^ Hypothetical protein FLJ22390 ^ | 0.82 |
| AGI_HUM1_OLIGO_A_23_P139575 | DKFZp564J157 ^ DKFZp564J157 protein ^ | 0.74 |
| AGI_HUM1_OLIGO_A_23_P126129 | C1orf25 ^ Chromosome 1 open reading frame 25 ^ | 0.61 |
| AGI_HUM1_OLIGO_A_23_P15937 | SMAD2 ^ SMAD, mothers against DPP homolog 2 (Drosophila) ^ | 0.75 |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_32_P20221 | SMARCE1 ^ SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1 ^ | 0.52 |
| AGI_HUM1_OLIGO_A_24_P867201 | ^ Homo sapiens, clone IMAGE:5277945, mRNA ^ | 0.35 |
| AGI_HUM1_OLIGO_A_23_P56978 | PTK6 ^ PTK6 protein tyrosine kinase 6 ^ | 0.31 |
| AGI_HUM1_OLIGO_A_23_P153958 | Uncharacterized gene | 0.59 |
| AGI_HUM1_OLIGO_A_23_P84475 | Uncharacterized gene | 0.64 |
| AGI_HUM1_OLIGO_A_24_P184230 | AASDHPPT ^ Aminoadipate-semialdehyde dehydrogenase-phosphopantetheinyl transferase ^ | 0.49 |
| AGI_HUM1_OLIGO_A_23_P350059 | ^ Similar to KIAA0454 protein ^ | 0.87 |
| AGI_HUM1_OLIGO_A_23_P32861 | CGI-07 ^ CGI-07 protein ^ | 0.49 |
| AGI_HUM1_OLIGO_A_23_P376449 | Uncharacterized gene | 0.68 |
| AGI_HUM1_OLIGO_A_24_P418250 | Uncharacterized gene | 0.62 |
| AGI_HUM1_OLIGO_A_23_P170058 | PSMB2 ^ Proteasome (prosome, macropain) subunit, beta type, 2 ^ | 0.73 |
| AGI_HUM1_OLIGO_A_23_P134854 | CLDN23 ^ Claudin 23 ^ | 0.63 |
| AGI_HUM1_OLIGO_A_23_P89755 | RNF138 ^ Ring finger protein 138 ^ | 0.73 |
| AGI_HUM1_OLIGO_A_23_P202532 | DKFZP566K0524 ^ DKFZP566K0524 protein ^ | 0.08 |
| AGI_HUM1_OLIGO_A_23_P73208 | MGC29891 ^ Hypothetical protein MGC29891 ^ | 0.44 |
| AGI_HUM1_OLIGO_A_23_P18304 | UBE1DC1 ^ Ubiquitin-activating enzyme E1-domain containing 1 ^ | 0.73 |
| AGI_HUM1_OLIGO_A_23_P361584 | FLJ32028 ^ Hypothetical protein FLJ32028 ^ | 0.46 |
| AGI_HUM1_OLIGO_A_32_P211752 | Uncharacterized gene | 0.65 |
| AGI_HUM1_OLIGO_A_24_P28622 | LOC90806 ^ Similar to RIKEN cDNA 2610307I21 ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P217938 | SPHAR ^ S-phase response (cyclin-related) ^ | 0.52 |
| AGI_HUM1_OLIGO_A_32_P3214 | Uncharacterized gene | 0.41 |
| | | |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P43484 | CDKN2A ^ Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4) ^ | 0.41 |
| AGI_HUM1_OLIGO_A_23_P12503 | NUP133 ^ Nucleoporin 133kDa ^ | 0.58 |
| AGI_HUM1_OLIGO_A_23_P11674 | PTGFR ^ Prostaglandin F receptor (FP) ^ | 0.18 |
| AGI_HUM1_OLIGO_A_23_P11295 | MTCP1 ^ Mature T-cell proliferation 1 ^ | 0.70 |
| AGI_HUM1_OLIGO_A_32_P16451 | SARA1 ^ SAR1a gene homolog 1 (S. cerevisiae) ^ | 0.49 |
| AGI_HUM1_OLIGO_A_23_P5742 | FLJ13646 ^ Hypothetical protein FLJ13646 ^ | 0.86 |
| AGI_HUM1_OLIGO_A_23_P208880 | UHRF1 ^ Ubiquitin-like, containing PHD and RING finger domains, 1 ^ | 0.35 |
| AGI_HUM1_OLIGO_A_23_P158725 | SLC16A3 ^ Solute carrier family 16 (monocarboxylic acid transporters), member 3 ^ | 0.22 |
| AGI_HUM1_OLIGO_A_23_P23815 | SLC30A1 ^ Solute carrier family 30 (zinc transporter), member 1 ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P133585 | CDK7 ^ Cyclin-dependent kinase 7 (MO15 homolog, Xenopus laevis, cdk-activating kinase) ^ | 0.71 |
| AGI_HUM1_OLIGO_A_23_P91487 | C21orf6 ^ Chromosome 21 open reading frame 6 ^ | 0.59 |
| AGI_HUM1_OLIGO_A_23_P167212 | QDPR ^ Quinoid dihydropteridine reductase ^ | 0.82 |
| AGI_HUM1_OLIGO_A_23_P59045 | HIST1H2AE ^ Histone 1, H2ae ^ | 0.75 |
| AGI_HUM1_OLIGO_A_24_P355816 | FLJ11259 ^ Hypothetical protein FLJ11259 ^ | 0.55 |
| AGI_HUM1_OLIGO_A_23_P23074 | IFI44 ^ Interferon-induced protein 44 ^ | 0.28 |
| AGI_HUM1_OLIGO_A_23_P32165 | LHX2 ^ LIM homeobox 2 ^ | 0.49 |
| AGI_HUM1_OLIGO_A_24_P59099 | Uncharacterized gene | 0.72 |
| AGI_HUM1_OLIGO_A_24_P364335 | OCIL ^ Lectin-like NK cell receptor ^ | 0.78 |
| AGI_HUM1_OLIGO_A_23_P111171 | B3GALT4 ^ UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 4 ^ | 0.53 |
| AGI_HUM1_OLIGO_A_24_P230037 | ^ CDNA clone IMAGE:3625232, partial cds ^ | 0.61 |
| AGI_HUM1_OLIGO_A_23_P123039 | NUPL2 ^ Nucleoporin like 2 ^ | 0.65 |
| | | |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P500421 | EYA2 ^ Eyes absent homolog 2 (Drosophila) ^ | 0.12 |
| AGI_HUM1_OLIGO_A_32_P199429 | Uncharacterized gene | 0.51 |
| AGI_HUM1_OLIGO_A_23_P119562 | DF ^ D component of complement (adipsin) ^ | 0.29 |
| AGI_HUM1_OLIGO_A_32_P11786 | SFRS6 ^ Splicing factor, arginine/serine-rich 6 ^ | 0.41 |
| AGI_HUM1_OLIGO_A_24_P175989 | VPS29 ^ Vacuolar protein sorting 29 (yeast) ^ | 0.82 |
| AGI_HUM1_OLIGO_A_23_P102202 | MSH6 ^ MutS homolog 6 (E. coli) ^ | 0.75 |
| AGI_HUM1_OLIGO_A_23_P399201 | TMSL3 ^ Thymosin-like 3 ^ | 0.19 |
| AGI_HUM1_OLIGO_A_23_P103486 | CYP2J2 ^ Cytochrome P450, family 2, subfamily J, polypeptide 2 ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P25030 | RODH ^ 3-hydroxysteroid epimerase ^ | 0.34 |
| AGI_HUM1_OLIGO_A_32_P132827 | Uncharacterized gene | 0.71 |
| AGI_HUM1_OLIGO_A_23_P23266 | Uncharacterized gene | 0.66 |
| AGI_HUM1_OLIGO_A_23_P98092 | OAT ^ Ornithine aminotransferase (gyrate atrophy) ^ | 0.55 |
| AGI_HUM1_OLIGO_A_24_P234094 | RAB7L1 ^ RAB7, member RAS oncogene family-like 1 ^ | 0.48 |
| AGI_HUM1_OLIGO_A_24_P229756 | Uncharacterized gene | 0.76 |
| AGI_HUM1_OLIGO_A_23_P19455 | MDC1 ^ Mediator of DNA damage checkpoint 1 ^ | 0.65 |
| AGI_HUM1_OLIGO_A_23_P35114 | CKIP-1 ^ CK2 interacting protein 1; HQ0024c protein ^ | 0.73 |
| AGI_HUM1_OLIGO_A_23_P310068 | Uncharacterized gene | 0.47 |
| AGI_HUM1_OLIGO_A_23_P106481 | LOC51234 ^ Hypothetical protein LOC51234 ^ | 0.75 |
| AGI_HUM1_OLIGO_A_24_P923142 | MGC14289 ^ Similar to RIKEN cDNA 1200014N16 gene ^ | 0.35 |
| AGI_HUM1_OLIGO_A_32_P5205 | Uncharacterized gene | 0.54 |
| AGI_HUM1_OLIGO_A_23_P252371 | RBBP8 ^ Retinoblastoma binding protein 8 ^ | 0.70 |
| AGI_HUM1_OLIGO_A_23_P121898 | PARP8 ^ Poly (ADP-ribose) polymerase family, member 8 ^ | 0.56 |
| AGI_HUM1_OLIGO_A_24_P89509 | Uncharacterized gene | 0.73 |
| | | |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P15305 | PRPSAP1 ^ Phosphoribosyl pyrophosphate synthetase-associated protein 1 ^ | 0.76 |
| AGI_HUM1_OLIGO_A_23_P60816 | PDCD6IP ^ Programmed cell death 6 interacting protein ^ | 0.68 |
| AGI_HUM1_OLIGO_A_23_P200710 | PIK3C2B ^ Phosphoinositide-3-kinase, class 2, beta polypeptide ^ | 0.84 |
| AGI_HUM1_OLIGO_A_32_P228124 | Uncharacterized gene | 0.48 |
| AGI_HUM1_OLIGO_A_23_P383986 | GALNAC4S-6ST ^ B cell RAG associated protein ^ | 0.41 |
| AGI_HUM1_OLIGO_A_23_P166280 | Uncharacterized gene | 0.50 |
| AGI_HUM1_OLIGO_A_23_P323685 | HIST1H4H ^ Histone 1, H4h ^ | 0.66 |
| AGI_HUM1_OLIGO_A_23_P8452 | Uncharacterized gene | 0.28 |
| AGI_HUM1_OLIGO_A_24_P935986 | BCAT1 ^ Branched chain aminotransferase 1, cytosolic ^ | 0.59 |
| AGI_HUM1_OLIGO_A_23_P216282 | ARHGEF10 ^ Rho guanine nucleotide exchange factor (GEF) 10 ^ | 0.69 |
| AGI_HUM1_OLIGO_A_24_P816844 | Uncharacterized gene | 0.49 |
| AGI_HUM1_OLIGO_A_24_P320221 | FLJ12716 ^ FLJ12716 protein ^ | 0.64 |
| AGI_HUM1_OLIGO_A_24_P228796 | GAGE2 ^ G antigen 7 ^ | 0.42 |
| AGI_HUM1_OLIGO_A_23_P29994 | RBPSUH ^ Recombining binding protein suppressor of hairless (Drosophila) ^ | 0.82 |
| AGI_HUM1_OLIGO_A_24_P165082 | ZNF222 ^ Zinc finger protein 222 ^ | 0.69 |
| AGI_HUM1_OLIGO_A_23_P203030 | DLAT ^ Dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex) ^ | 0.54 |
| AGI_HUM1_OLIGO_A_32_P223256 | Uncharacterized gene | 0.72 |
| AGI_HUM1_OLIGO_A_23_P166248 | DSCR1 ^ Down syndrome critical region gene 1 ^ | 0.51 |
| AGI_HUM1_OLIGO_A_23_P5912 | YTHDF1 ^ YTH domain family, member 1 ^ | 0.73 |
| AGI_HUM1_OLIGO_A_23_P134113 | C6orf192 ^ Chromosome 6 open reading frame 192 ^ | 0.48 |
| AGI_HUM1_OLIGO_A_23_P37244 | SNAPC1 ^ Small nuclear RNA activating complex, polypeptide 1, 43kDa ^ | 0.72 |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P99253 | LIN7A ^ Lin-7 homolog A (C. elegans) ^ | 0.50 |
| AGI_HUM1_OLIGO_A_23_P87049 | SORL1 ^ Sortilin-related receptor, L(DLR class) A repeats-containing ^ | 0.57 |
| AGI_HUM1_OLIGO_A_24_P229616 | Uncharacterized gene | 0.62 |
| AGI_HUM1_OLIGO_A_32_P182473 | ZNF625 ^ Zinc finger protein 625 ^ | 0.66 |
| AGI_HUM1_OLIGO_A_24_P147263 | USP31 ^ Ubiquitin specific protease 31 ^ | 0.60 |
| AGI_HUM1_OLIGO_A_24_P498854 | LOC284998 ^ Hypothetical protein LOC284998 ^ | 0.62 |
| AGI_HUM1_OLIGO_A_24_P236799 | RAB31 ^ RAB31, member RAS oncogene family ^ | 0.38 |
| AGI_HUM1_OLIGO_A_24_P924484 | Uncharacterized gene | 0.59 |
| AGI_HUM1_OLIGO_A_23_P319859 | EYA2 ^ Eyes absent homolog 2 (Drosophila) ^ | 0.20 |
| AGI_HUM1_OLIGO_A_24_P90022 | Uncharacterized gene | 0.69 |
| AGI_HUM1_OLIGO_A_23_P27265 | C18orf4 ^ Chromosome 18 open reading frame 4 ^ | 0.33 |
| AGI_HUM1_OLIGO_A_23_P52806 | BACE1 ^ Beta-site APP-cleaving enzyme 1 ^ | 0.55 |
| AGI_HUM1_OLIGO_A_23_P357811 | MBNL1 ^ Muscleblind-like (Drosophila) ^ | 0.65 |
| AGI_HUM1_OLIGO_A_32_P224911 | TATDN1 ^ TatD DNase domain containing 1 ^ | 0.69 |
| AGI_HUM1_OLIGO_A_23_P11652 | USP1 ^ Ubiquitin specific protease 1 ^ | 0.92 |
| AGI_HUM1_OLIGO_A_23_P131394 | SLC16A14 ^ Solute carrier family 16 (monocarboxylic acid transporters), member 14 ^ | 0.28 |
| AGI_HUM1_OLIGO_A_23_P717 | FLJ10874 ^ Hypothetical protein FLJ10874 ^ | 0.58 |
| AGI_HUM1_OLIGO_A_23_P45934 | SRP9 ^ Signal recognition particle 9kDa ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P155316 | NCBP2 ^ Nuclear cap binding protein subunit 2, 20kDa ^ | 0.85 |
| AGI_HUM1_OLIGO_A_23_P134147 | ZBTB24 ^ Zinc finger and BTB domain containing 24 ^ | 0.70 |
| AGI_HUM1_OLIGO_A_24_P62530 | RHOU ^ Ras homolog gene family, member U ^ | 0.69 |
| AGI_HUM1_OLIGO_A_24_P106591 | TXNDC5 ^ Thioredoxin domain containing 5 ^ | 0.47 |
| AGI_HUM1_OLIGO_A_23_P166087 | RASSF2 ^ Ras association (RalGDS/AF-6) domain family 2 ^ | 0.69 |

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_32_P99100 | PTPRK ^ Protein tyrosine phosphatase, receptor type, K ^ | 0.50 |

**Table 5.3. The list of the genes that are significantly up regulated in FL-Y267F-AR compared to vector control.**

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P111506 | Uncharacterized gene | 1.49 |
| AGI_HUM1_OLIGO_A_23_P304530 | LCE5A ^ Late cornified envelope 5A ^ | 1.49 |
| AGI_HUM1_OLIGO_A_24_P508410 | LOC388524 ^ Similar to Laminin receptor 1 ^ | 1.72 |
| AGI_HUM1_OLIGO_A_23_P125519 | RPS4X ^ Ribosomal protein S4, X-linked ^ | 1.10 |
| AGI_HUM1_OLIGO_A_23_P115118 | BMP8A ^ Bone morphogenetic protein 8a ^ | 1.39 |
| AGI_HUM1_OLIGO_A_32_P90455 | Uncharacterized gene | 1.34 |
| AGI_HUM1_OLIGO_A_32_P112100 | Uncharacterized gene | 1.48 |
| AGI_HUM1_OLIGO_A_23_P130194 | PYCR1 ^ Pyrroline-5-carboxylate reductase 1 ^ | 1.30 |
| AGI_HUM1_OLIGO_A_23_P431853 | Uncharacterized gene | 2.23 |
| AGI_HUM1_OLIGO_A_24_P279060 | HCN4 ^ Hyperpolarization activated cyclic nucleotide-gated potassium channel 4 ^ | 1.34 |
| AGI_HUM1_OLIGO_A_24_P203909 | RPL34 ^ Ribosomal protein L34 ^ | 1.10 |
| AGI_HUM1_OLIGO_A_32_P435367 | PSMD4 ^ Proteasome (prosome, macropain) 26S subunit, non-ATPase, 4 ^ | 1.32 |

**Table 5.4. The list of the genes that are significantly up regulated in TR-WT-AR compared to vector control.**

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P221057 | Uncharacterized gene | 1.40 |
| AGI_HUM1_OLIGO_A_23_P76823 | ADSSL1 ^ Adenylosuccinate synthase like 1 ^ | 1.73 |
| AGI_HUM1_OLIGO_A_23_P372234 | CA12 ^ Carbonic anhydrase XII ^ | 3.01 |
| AGI_HUM1_OLIGO_A_23_P374389 | PWWP2 ^ PWWP domain containing 2 ^ | 1.17 |
| AGI_HUM1_OLIGO_A_23_P159544 | ASMTL ^ Acetylserotonin O-methyltransferase-like ^ | 1.92 |
| AGI_HUM1_OLIGO_A_24_P926820 | Uncharacterized gene | 1.62 |
| AGI_HUM1_OLIGO_A_23_P138899 | COPB ^ Coatomer protein complex, subunit beta ^ | 1.46 |
| AGI_HUM1_OLIGO_A_24_P58477 | Uncharacterized gene | 1.42 |
| AGI_HUM1_OLIGO_A_23_P37983 | MT1B ^ Metallothionein 1B (functional) ^ | 2.26 |
| AGI_HUM1_OLIGO_A_32_P150856 | Uncharacterized gene | 1.46 |
| AGI_HUM1_OLIGO_A_23_P373819 | TUSC1 ^ Tumor suppressor candidate 1 ^ | 2.00 |
| AGI_HUM1_OLIGO_A_32_P21384 | RPL17 ^ Ribosomal protein L17 ^ | 1.30 |
| AGI_HUM1_OLIGO_A_23_P56069 | C19orf13 ^ Chromosome 19 open reading frame 13 ^ | 1.38 |
| AGI_HUM1_OLIGO_A_23_P138706 | ADRA2A ^ Adrenergic, alpha-2A-, receptor ^ | 2.81 |
| AGI_HUM1_OLIGO_A_23_P11800 | CaMKIINalpha ^ Calcium/calmodulin-dependent protein kinase II ^ | 4.36 |
| AGI_HUM1_OLIGO_A_23_P104624 | Uncharacterized gene | 1.96 |
| AGI_HUM1_OLIGO_A_23_P255663 | MANEA ^ Mannosidase, endo-alpha ^ | 2.74 |
| AGI_HUM1_OLIGO_A_24_P260101 | MME ^ Membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase, CALLA, CD10) ^ | 3.16 |
| AGI_HUM1_OLIGO_A_24_P827738 | Uncharacterized gene | 1.63 |
| AGI_HUM1_OLIGO_A_24_P398319 | C11orf15 ^ Chromosome 11 open reading frame 15 ^ | 1.39 |
| AGI_HUM1_OLIGO_A_23_P252306 | ID1 ^ Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein ^ | 2.85 |
| AGI_HUM1_OLIGO_A_24_P332953 | Uncharacterized gene | 3.53 |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P143676 | Uncharacterized gene | 1.46 |
| AGI_HUM1_OLIGO_A_24_P263672 | LOC96610 ^ Hypothetical protein similar to KIAA0187 gene product ^ | 1.57 |
| AGI_HUM1_OLIGO_A_23_P414343 | MT1H ^ Metallothionein 1H ^ | 2.12 |
| AGI_HUM1_OLIGO_A_23_P252413 | Uncharacterized gene | 2.01 |
| AGI_HUM1_OLIGO_A_23_P121869 | Uncharacterized gene | 1.67 |
| AGI_HUM1_OLIGO_A_23_P81859 | HIST1H2AH ^ Histone 1, H2ah ^ | 2.60 |
| AGI_HUM1_OLIGO_A_24_P361896 | MT2A ^ Metallothionein 2A ^ | 2.13 |
| AGI_HUM1_OLIGO_A_32_P164215 | Uncharacterized gene | 1.80 |
| AGI_HUM1_OLIGO_A_24_P135322 | NRP1 ^ Neuropilin 1 ^ | 1.63 |
| AGI_HUM1_OLIGO_A_23_P372516 | TCF8 ^ Transcription factor 8 (represses interleukin 2 expression) ^ | 1.09 |
| AGI_HUM1_OLIGO_A_23_P33809 | C15orf12 ^ Chromosome 15 open reading frame 12 ^ | 1.21 |
| AGI_HUM1_OLIGO_A_23_P39131 | GLTSCR2 ^ Glioma tumor suppressor candidate region gene 2 ^ | 1.29 |
| AGI_HUM1_OLIGO_A_24_P347624 | SNRPN ^ SNRPN upstream reading frame ^ | 1.35 |
| AGI_HUM1_OLIGO_A_23_P42575 | CALD1 ^ Caldesmon 1 ^ | 1.76 |
| AGI_HUM1_OLIGO_A_24_P573978 | Uncharacterized gene | 1.20 |
| AGI_HUM1_OLIGO_A_24_P201323 | ZMYND11 ^ Zinc finger, MYND domain containing 11 ^ | 1.82 |
| AGI_HUM1_OLIGO_A_23_P354170 | FLJ20522 ^ GPI-mannosyltransferase subunit ^ | 1.52 |
| AGI_HUM1_OLIGO_A_23_P163782 | MT1H ^ Metallothionein 1H ^ | 2.12 |
| AGI_HUM1_OLIGO_A_23_P153256 | MGC4728 ^ Similar to hypothetical protein FLJ23233 ^ | 1.79 |
| AGI_HUM1_OLIGO_A_23_P168014 | HIST1H2AJ ^ Histone 1, H2aj ^ | 1.60 |
| AGI_HUM1_OLIGO_A_24_P230176 | LOC339229 ^ Hypothetical protein LOC339229 ^ | 1.69 |
| AGI_HUM1_OLIGO_A_24_P133475 | DHRS1 ^ Dehydrogenase/reductase (SDR family) member 1 ^ | 1.24 |
| AGI_HUM1_OLIGO_A_24_P145787 | Uncharacterized gene | 1.12 |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P391368 | ATXN10 ^ Ataxin 10 ^ | 1.29 |
| AGI_HUM1_OLIGO_A_24_P261724 | RNF10 ^ Ring finger protein 10 ^ | 1.10 |
| AGI_HUM1_OLIGO_A_23_P34800 | NASP ^ Nuclear autoantigenic sperm protein (histone-binding) ^ | 1.30 |
| AGI_HUM1_OLIGO_A_23_P96853 | FAF1 ^ Fas (TNFRSF6) associated factor 1 ^ | 1.33 |
| AGI_HUM1_OLIGO_A_23_P70045 | H2AFY ^ H2A histone family, member Y ^ | 1.77 |
| AGI_HUM1_OLIGO_A_24_P508410 | LOC388524 ^ Similar to Laminin receptor 1 ^ | 1.85 |
| AGI_HUM1_OLIGO_A_32_P152986 | Uncharacterized gene | 1.61 |
| AGI_HUM1_OLIGO_A_23_P47614 | PHLDA2 ^ Pleckstrin homology-like domain, family A, member 2 ^ | 1.67 |
| AGI_HUM1_OLIGO_A_23_P57521 | EIF3S6IP ^ Eukaryotic translation initiation factor 3, subunit 6 interacting protein ^ | 1.54 |
| AGI_HUM1_OLIGO_A_23_P310274 | PRSS2 ^ Protease, serine, 2 (trypsin 2) ^ | 6.48 |
| AGI_HUM1_OLIGO_A_23_P135437 | SEC8L1 ^ SEC8-like 1 (S. cerevisiae) ^ | 1.40 |
| AGI_HUM1_OLIGO_A_24_P161086 | NFKBIB ^ Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta ^ | 1.74 |
| AGI_HUM1_OLIGO_A_23_P27306 | COLEC12 ^ Collectin sub-family member 12 ^ | 1.75 |
| AGI_HUM1_OLIGO_A_23_P17786 | PITPNB ^ Phosphatidylinositol transfer protein, beta ^ | 1.55 |
| AGI_HUM1_OLIGO_A_24_P363615 | MTPN ^ Myotrophin ^ | 1.96 |
| AGI_HUM1_OLIGO_A_24_P84408 | Uncharacterized gene | 1.24 |
| AGI_HUM1_OLIGO_A_23_P322845 | HTPAP ^ HTPAP protein ^ | 1.57 |
| AGI_HUM1_OLIGO_A_24_P125096 | MT1X ^ Metallothionein 1X ^ | 2.11 |
| AGI_HUM1_OLIGO_A_32_P181222 | KCNMA1 ^ Potassium large conductance calcium-activated channel, subfamily M, alpha member 1 ^ | 2.96 |
| AGI_HUM1_OLIGO_A_24_P551302 | RARA ^ Retinoic acid receptor, alpha ^ | 1.36 |
| AGI_HUM1_OLIGO_A_24_P341408 | Uncharacterized gene | 1.21 |
| AGI_HUM1_OLIGO_A_32_P135385 | Uncharacterized gene | 1.30 |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P48057 | IRX5 ^ Iroquois homeobox protein 5 ^ | 1.86 |
| AGI_HUM1_OLIGO_A_24_P86389 | HIST1H2AM ^ Histone 1, H2am ^ | 1.85 |
| AGI_HUM1_OLIGO_A_24_P918808 | OAZ1 ^ Ornithine decarboxylase antizyme 1 ^ | 1.98 |
| AGI_HUM1_OLIGO_A_32_P227845 | ^ CDNA clone IMAGE:3346533, containing frame-shift errors ^ | 1.24 |
| AGI_HUM1_OLIGO_A_23_P54840 | MT1A ^ Metallothionein 1A (functional) ^ | 1.91 |
| AGI_HUM1_OLIGO_A_32_P191290 | ^ Transcribed locus ^ | 1.53 |
| AGI_HUM1_OLIGO_A_24_P345081 | AE2 ^ Hypothetical protein AE2 ^ | 1.57 |
| AGI_HUM1_OLIGO_A_23_P60286 | EIF4B ^ Eukaryotic translation initiation factor 4B ^ | 1.25 |
| AGI_HUM1_OLIGO_A_23_P115460 | RPL22 ^ Ribosomal protein L22 ^ | 1.17 |
| AGI_HUM1_OLIGO_A_23_P23194 | PINK1 ^ PTEN induced putative kinase 1 ^ | 1.75 |
| AGI_HUM1_OLIGO_A_24_P353289 | C22orf13 ^ Chromosome 22 open reading frame 13 ^ | 1.32 |
| AGI_HUM1_OLIGO_A_23_P55716 | LU ^ Lutheran blood group (Auberger b antigen included) ^ | 1.43 |
| AGI_HUM1_OLIGO_A_24_P285880 | TLOC1 ^ Translocation protein 1 ^ | 1.39 |
| AGI_HUM1_OLIGO_A_23_P48570 | DHRS2 ^ Dehydrogenase/reductase (SDR family) member 2 ^ | 3.34 |
| AGI_HUM1_OLIGO_A_23_P15174 | MT1F ^ Metallothionein 1F (functional) ^ | 2.15 |
| AGI_HUM1_OLIGO_A_23_P250035 | SDHA ^ Succinate dehydrogenase complex, subunit A, flavoprotein (Fp) ^ | 1.27 |
| AGI_HUM1_OLIGO_A_23_P56810 | SLC4A1AP ^ Solute carrier family 4 (anion exchanger), member 1, adaptor protein ^ | 1.32 |
| AGI_HUM1_OLIGO_A_32_P194072 | DKFZP434B0335 ^ DKFZP434B0335 protein ^ | 1.74 |
| AGI_HUM1_OLIGO_A_24_P753476 | Uncharacterized gene | 1.55 |
| AGI_HUM1_OLIGO_A_23_P61881 | ARIH2 ^ Ariadne homolog 2 (Drosophila) ^ | 1.45 |
| AGI_HUM1_OLIGO_A_23_P106016 | PRKD1 ^ Protein kinase D1 ^ | 1.43 |
| | | |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_32_P67259 | SDHA ^ Succinate dehydrogenase complex, subunit A, flavoprotein (Fp) ^ | 1.24 |
| AGI_HUM1_OLIGO_A_23_P16110 | Uncharacterized gene | 1.38 |

**Table 5.5. The list of the genes that are significantly down regulated in TR-WT-AR compared to vector control.**

| Agilent Gene ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P103698 | MAPBPIP ^ Mitogen-activated protein-binding protein-interacting protein ^ | 0.59 |
| AGI_HUM1_OLIGO_A_32_P146635 | SESN3 ^ Sestrin 3 ^ | 0.39 |
| AGI_HUM1_OLIGO_A_32_P119830 | PEG3 ^ Paternally expressed 3 ^ | 0.21 |
| AGI_HUM1_OLIGO_A_23_P200386 | KHDRBS1 ^ KH domain containing, RNA binding, signal transduction associated 1 ^ | 0.75 |
| AGI_HUM1_OLIGO_A_23_P4821 | JUNB ^ Jun B proto-oncogene ^ | 0.76 |
| AGI_HUM1_OLIGO_A_24_P364335 | OCIL ^ Lectin-like NK cell receptor ^ | 0.83 |
| AGI_HUM1_OLIGO_A_23_P337168 | FLJ30596 ^ Hypothetical protein FLJ30596 ^ | 0.48 |
| AGI_HUM1_OLIGO_A_23_P114783 | PARP1 ^ Poly (ADP-ribose) polymerase family, member 1 ^ | 0.51 |
| AGI_HUM1_OLIGO_A_23_P141362 | FZD2 ^ Frizzled homolog 2 (Drosophila) ^ | 0.22 |
| AGI_HUM1_OLIGO_A_23_P214046 | FBXW11 ^ F-box and WD-40 domain protein 11 ^ | 0.38 |
| AGI_HUM1_OLIGO_A_23_P73589 | MSN ^ Moesin ^ | 0.53 |
| AGI_HUM1_OLIGO_A_32_P33576 | OPRK1 ^ Opioid receptor, kappa 1 ^ | 0.22 |
| AGI_HUM1_OLIGO_A_23_P34375 | TCEA3 ^ Transcription elongation factor A (SII), 3 ^ | 0.17 |
| AGI_HUM1_OLIGO_A_24_P302172 | PTGFR ^ Prostaglandin F receptor (FP) ^ | 0.11 |
| AGI_HUM1_OLIGO_A_23_P66777 | CDC27 ^ Cell division cycle 27 ^ | 0.46 |
| AGI_HUM1_OLIGO_A_23_P52189 | GGPS1 ^ Geranylgeranyl diphosphate synthase 1 ^ | 0.61 |
| AGI_HUM1_OLIGO_A_23_P254271 | TUBB6 ^ Tubulin, beta 6 ^ | 0.07 |
| AGI_HUM1_OLIGO_A_24_P186944 | Uncharacterized gene | 0.67 |
| AGI_HUM1_OLIGO_A_24_P521994 | DRE1 ^ DRE1 protein ^ | 0.45 |
| AGI_HUM1_OLIGO_A_24_P255516 | UNQ6077 ^ Similar to testis-specific protein NYD-TSP1 ^ | 0.56 |
| AGI_HUM1_OLIGO_A_23_P309361 | FLJ30525 ^ Hypothetical protein FLJ30525 ^ | 0.02 |
| AGI_HUM1_OLIGO_A_24_P857404 | LOC92312 ^ Hypothetical protein LOC92312 ^ | 0.47 |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P42087 | BPHL ^ Biphenyl hydrolase-like (serine hydrolase; breast epithelial mucin-associated antigen) ^ | 0.57 |
| AGI_HUM1_OLIGO_A_32_P166921 | TK2 ^ Thymidine kinase 2, mitochondrial ^ | 0.65 |
| AGI_HUM1_OLIGO_A_23_P143147 | TCFL5 ^ Transcription factor-like 5 (basic helix-loop-helix) ^ | 0.61 |
| AGI_HUM1_OLIGO_A_24_P839239 | Uncharacterized gene | 0.43 |
| AGI_HUM1_OLIGO_A_23_P76538 | TSC ^ Hypothetical protein FLJ20607 ^ | 0.36 |
| AGI_HUM1_OLIGO_A_23_P212968 | UGT2B11 ^ UDP glycosyltransferase 2 family, polypeptide B11 ^ | 0.64 |
| AGI_HUM1_OLIGO_A_24_P62530 | RHOU ^ Ras homolog gene family, member U ^ | 0.74 |
| AGI_HUM1_OLIGO_A_23_P160406 | KCTD3 ^ Potassium channel tetramerisation domain containing 3 ^ | 0.65 |
| AGI_HUM1_OLIGO_A_23_P25684 | RDH11 ^ DKFZP564M1462 protein ^ | 0.59 |
| AGI_HUM1_OLIGO_A_24_P365571 | MRPS6 ^ Mitochondrial ribosomal protein S6 ^ | 0.50 |
| AGI_HUM1_OLIGO_A_23_P208706 | BAX ^ BCL2-associated X protein ^ | 0.58 |
| AGI_HUM1_OLIGO_A_23_P116235 | Uncharacterized gene | 0.12 |
| AGI_HUM1_OLIGO_A_23_P160546 | FLJ11280 ^ Hypothetical protein FLJ11280 ^ | 0.24 |
| AGI_HUM1_OLIGO_A_23_P412577 | ANKRD29 ^ Ankyrin repeat domain 29 ^ | 0.19 |
| AGI_HUM1_OLIGO_A_23_P82206 | ECHDC1 ^ Enoyl Coenzyme A hydratase domain containing 1 ^ | 0.06 |
| AGI_HUM1_OLIGO_A_23_P85952 | FLJ22457 ^ Hypothetical protein FLJ22457 ^ | 0.09 |
| AGI_HUM1_OLIGO_A_23_P68106 | TMSB10 ^ Thymosin, beta 10 ^ | 0.37 |
| AGI_HUM1_OLIGO_A_24_P84428 | CACYBP ^ Calcyclin binding protein ^ | 0.80 |
| AGI_HUM1_OLIGO_A_23_P11295 | MTCP1 ^ Mature T-cell proliferation 1 ^ | 0.69 |
| AGI_HUM1_OLIGO_A_23_P137578 | FBXO28 ^ F-box protein 28 ^ | 0.74 |
| AGI_HUM1_OLIGO_A_23_P46507 | CGI-143 ^ CGI-143 protein ^ | 0.47 |
| AGI_HUM1_OLIGO_A_23_P44291 | CRTAP ^ Cartilage associated protein ^ | 0.65 |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P405205 | ATP2B4 ^ ATPase, Ca++ transporting, plasma membrane 4 ^ | 0.33 |
| AGI_HUM1_OLIGO_A_24_P413126 | TMEPAI ^ Transmembrane, prostate androgen induced RNA ^ | 0.72 |
| AGI_HUM1_OLIGO_A_23_P131394 | SLC16A14 ^ Solute carrier family 16 (monocarboxylic acid transporters), member 14 ^ | 0.29 |
| AGI_HUM1_OLIGO_A_23_P88484 | DUT ^ DUTP pyrophosphatase ^ | 0.85 |
| AGI_HUM1_OLIGO_A_23_P43946 | CIP29 ^ Cytokine induced protein 29 kDa ^ | 0.65 |
| AGI_HUM1_OLIGO_A_23_P18518 | TRAM1L1 ^ Translocation associated membrane protein 1-like 1 ^ | 0.43 |
| AGI_HUM1_OLIGO_A_23_P374844 | GAL ^ Galanin ^ | 0.44 |
| AGI_HUM1_OLIGO_A_23_P83438 | FLJ13855 ^ Hypothetical protein FLJ13855 ^ | 0.59 |
| AGI_HUM1_OLIGO_A_24_P384990 | ^ Homo sapiens, clone IMAGE:4157625, mRNA ^ | 0.45 |
| AGI_HUM1_OLIGO_A_23_P39445 | RKHD1 ^ Ring finger and KH domain containing 1 ^ | 0.25 |
| AGI_HUM1_OLIGO_A_23_P399201 | TMSL3 ^ Thymosin-like 3 ^ | 0.17 |
| AGI_HUM1_OLIGO_A_24_P382017 | RRN3 ^ RRN3 RNA polymerase I transcription factor homolog (yeast) ^ | 0.59 |
| AGI_HUM1_OLIGO_A_23_P200507 | HSPC163 ^ HSPC163 protein ^ | 0.58 |
| AGI_HUM1_OLIGO_A_23_P119562 | DF ^ D component of complement (adipsin) ^ | 0.25 |
| AGI_HUM1_OLIGO_A_24_P849245 | Uncharacterized gene | 0.47 |
| AGI_HUM1_OLIGO_A_24_P366495 | Uncharacterized gene | 0.67 |
| AGI_HUM1_OLIGO_A_23_P103414 | YTHDF2 ^ YTH domain family, member 2 ^ | 0.66 |
| AGI_HUM1_OLIGO_A_23_P112481 | AQP3 ^ Aquaporin 3 ^ | 0.30 |
| AGI_HUM1_OLIGO_A_24_P703614 | Uncharacterized gene | 0.61 |
| AGI_HUM1_OLIGO_A_23_P59791 | LUC7L2 ^ LUC7-like 2 (S. cerevisiae) ^ | 0.56 |
| AGI_HUM1_OLIGO_A_32_P171061 | ASCL2 ^ Achaete-scute complex-like 2 (Drosophila) ^ | 0.16 |
| AGI_HUM1_OLIGO_A_23_P52082 | DKFZP434B168 ^ DKFZP434B168 protein ^ | 0.65 |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| | | |
| AGI_HUM1_OLIGO_A_23_P21033 | GMPS ^ Guanine monphosphate synthetase ^ | 0.85 |
| AGI_HUM1_OLIGO_A_23_P145408 | FUCA2 ^ Fucosidase, alpha-L- 2, plasma ^ | 0.05 |
| AGI_HUM1_OLIGO_A_24_P4212 | PYCRL ^ Pyrroline-5-carboxylate reductase-like ^ | 0.73 |
| AGI_HUM1_OLIGO_A_24_P374513 | Uncharacterized gene | 0.23 |
| AGI_HUM1_OLIGO_A_24_P304051 | GSTO1 ^ Glutathione S-transferase omega 1 ^ | 0.75 |
| AGI_HUM1_OLIGO_A_23_P256735 | PGCP ^ Plasma glutamate carboxypeptidase ^ | 0.33 |
| AGI_HUM1_OLIGO_A_23_P128991 | C14orf156 ^ Chromosome 14 open reading frame 156 ^ | 0.73 |
| AGI_HUM1_OLIGO_A_23_P161190 | VIM ^ Vimentin ^ | 0.11 |
| AGI_HUM1_OLIGO_A_23_P49546 | GRIN2C ^ Glutamate receptor, ionotropic, N-methyl D-aspartate 2C ^ | 0.17 |
| AGI_HUM1_OLIGO_A_23_P52278 | KIF11 ^ Kinesin family member 11 ^ | 0.61 |
| AGI_HUM1_OLIGO_A_24_P230009 | Uncharacterized gene | 0.67 |
| AGI_HUM1_OLIGO_A_23_P19389 | HMGN4 ^ High mobility group nucleosomal binding domain 4 ^ | 0.70 |
| AGI_HUM1_OLIGO_A_23_P24633 | THY28 ^ Thymocyte protein thy28 ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P202696 | KBTBD4 ^ Kelch repeat and BTB (POZ) domain containing 4 ^ | 0.72 |
| AGI_HUM1_OLIGO_A_23_P255952 | MYO6 ^ Myosin VI ^ | 0.40 |
| AGI_HUM1_OLIGO_A_23_P85742 | CREG1 ^ Cellular repressor of E1A-stimulated genes 1 ^ | 0.74 |
| AGI_HUM1_OLIGO_A_23_P132718 | SEMA3B ^ Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B ^ | 0.74 |
| AGI_HUM1_OLIGO_A_23_P356554 | BAG2 ^ BCL2-associated athanogene 2 ^ | 0.23 |
| AGI_HUM1_OLIGO_A_24_P327886 | TCEA3 ^ Transcription elongation factor A (SII), 3 ^ | 0.08 |
| AGI_HUM1_OLIGO_A_32_P26443 | Uncharacterized gene | 0.38 |
| AGI_HUM1_OLIGO_A_24_P83262 | TM4SF6 ^ Transmembrane 4 superfamily member 6 ^ | 0.29 |
| AGI_HUM1_OLIGO_A_32_P22263 | HMGN1 ^ High-mobility group nucleosome binding domain 1 ^ | 0.67 |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P245246 | PIP5K2B ^ Phosphatidylinositol-4-phosphate 5-kinase, type II, beta ^ | 0.78 |
| AGI_HUM1_OLIGO_A_32_P137632 | FBXL17 ^ F-box and leucine-rich repeat protein 17 ^ | 0.35 |
| AGI_HUM1_OLIGO_A_23_P403335 | SLAC2-B ^ SLAC2-B ^ | 0.32 |
| AGI_HUM1_OLIGO_A_23_P1594 | VEGFB ^ Vascular endothelial growth factor B ^ | 0.60 |
| AGI_HUM1_OLIGO_A_24_P65597 | RPS19 ^ Ribosomal protein S19 ^ | 0.47 |
| AGI_HUM1_OLIGO_A_23_P201400 | PPOX ^ Protoporphyrinogen oxidase ^ | 0.70 |
| AGI_HUM1_OLIGO_A_24_P521559 | UGT2B10 ^ UDP glycosyltransferase 2 family, polypeptide B10 ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P48585 | SALL2 ^ Sal-like 2 (Drosophila) ^ | 0.31 |
| AGI_HUM1_OLIGO_A_24_P115621 | Uncharacterized gene | 0.60 |
| AGI_HUM1_OLIGO_A_32_P231617 | TM4SF1 ^ Transmembrane 4 superfamily member 1 ^ | 0.43 |
| AGI_HUM1_OLIGO_A_23_P158725 | SLC16A3 ^ Solute carrier family 16 (monocarboxylic acid transporters), member 3 ^ | 0.24 |
| AGI_HUM1_OLIGO_A_23_P202532 | DKFZP566K0524 ^ DKFZP566K0524 protein ^ | 0.19 |
| AGI_HUM1_OLIGO_A_23_P251795 | GPC2 ^ Glypican 2 (cerebroglycan) ^ | 0.56 |
| AGI_HUM1_OLIGO_A_23_P343382 | Uncharacterized gene | 0.21 |
| AGI_HUM1_OLIGO_A_23_P94118 | GTF2E2 ^ General transcription factor IIE, polypeptide 2, beta 34kDa ^ | 0.83 |
| AGI_HUM1_OLIGO_A_23_P377819 | SFRS5 ^ Splicing factor, arginine/serine-rich 5 ^ | 0.84 |
| AGI_HUM1_OLIGO_A_24_P204971 | HBP1 ^ HMG-box transcription factor 1 ^ | 0.64 |
| AGI_HUM1_OLIGO_A_23_P258048 | ZKSCAN1 ^ Zinc finger with KRAB and SCAN domains 1 ^ | 0.78 |
| AGI_HUM1_OLIGO_A_23_P211007 | NRIP1 ^ Nuclear receptor interacting protein 1 ^ | 0.49 |
| AGI_HUM1_OLIGO_A_32_P138004 | FAM45A ^ Family with sequence similarity 45, member A ^ | 0.45 |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P200930 | MTR ^ 5-methyltetrahydrofolate-homocysteine methyltransferase ^ | 0.57 |
| AGI_HUM1_OLIGO_A_32_P208424 | H3F3A ^ H3 histone, family 3A ^ | 0.52 |
| AGI_HUM1_OLIGO_A_23_P70371 | PHF3 ^ PHD finger protein 3 ^ | 0.53 |
| AGI_HUM1_OLIGO_A_23_P6963 | UBE2E1 ^ Ubiquitin-conjugating enzyme E2E 1 (UBC4/5 homolog, yeast) ^ | 0.24 |
| AGI_HUM1_OLIGO_A_23_P171143 | TM4SF6 ^ Transmembrane 4 superfamily member 6 ^ | 0.30 |
| AGI_HUM1_OLIGO_A_23_P62764 | MGC1203 ^ Hypothetical protein MGC1203 ^ | 0.52 |
| AGI_HUM1_OLIGO_A_23_P163858 | ZNRF1 ^ Zinc and ring finger 1 ^ | 0.59 |
| AGI_HUM1_OLIGO_A_24_P358578 | Uncharacterized gene | 0.61 |
| AGI_HUM1_OLIGO_A_24_P54863 | FLJ39370 ^ Hypothetical protein FLJ39370 ^ | 0.77 |
| AGI_HUM1_OLIGO_A_23_P67708 | TCF3 ^ Transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47) ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P97952 | C10orf125 ^ Chromosome 10 open reading frame 125 ^ | 0.71 |
| AGI_HUM1_OLIGO_A_32_P5251 | RARA ^ Retinoic acid receptor, alpha ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P206359 | CDH1 ^ Cadherin 1, type 1, E-cadherin (epithelial) ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P211047 | BACH1 ^ BTB and CNC homology 1, basic leucine zipper transcription factor 1 ^ | 0.64 |
| AGI_HUM1_OLIGO_A_23_P137984 | S100A10 ^ S100 calcium binding protein A10 (annexin II ligand, calpactin I, light polypeptide (p11)) ^ | 0.05 |
| AGI_HUM1_OLIGO_A_24_P923142 | MGC14289 ^ Similar to RIKEN cDNA 1200014N16 gene ^ | 0.28 |
| AGI_HUM1_OLIGO_A_32_P46840 | IL17D ^ Interleukin 17D ^ | 0.77 |
| AGI_HUM1_OLIGO_A_24_P374516 | TMSB4X ^ Thymosin, beta 4, X-linked ^ | 0.23 |
| AGI_HUM1_OLIGO_A_23_P2181 | CYB5R2 ^ Cytochrome b5 reductase b5R.2 ^ | 0.56 |
| AGI_HUM1_OLIGO_A_23_P155765 | HMGB2 ^ High-mobility group box 2 ^ | 0.63 |
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| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P228796 | GAGE2 ^ G antigen 7 ^ | 0.47 |
| AGI_HUM1_OLIGO_A_24_P104174 | TAOK3 ^ TAO kinase 3 ^ | 0.43 |
| AGI_HUM1_OLIGO_A_23_P162874 | HSPCA ^ Heat shock 90kDa protein 1, alpha ^ | 0.58 |
| AGI_HUM1_OLIGO_A_23_P350886 | KIAA0240 ^ KIAA0240 ^ | 0.46 |
| AGI_HUM1_OLIGO_A_23_P143981 | FBLN2 ^ Fibulin 2 ^ | 0.33 |
| AGI_HUM1_OLIGO_A_23_P43490 | CDKN2A ^ Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4) ^ | 0.58 |
| AGI_HUM1_OLIGO_A_23_P254288 | Uncharacterized gene | 0.80 |
| AGI_HUM1_OLIGO_A_23_P215421 | DKFZP434J154 ^ WIPI49-like protein 2 ^ | 0.56 |
| AGI_HUM1_OLIGO_A_24_P310756 | Uncharacterized gene | 0.85 |
| AGI_HUM1_OLIGO_A_23_P215956 | MYC ^ V-myc myelocytomatosis viral oncogene homolog (avian) ^ | 0.51 |
| AGI_HUM1_OLIGO_A_23_P63999 | Uncharacterized gene | 0.65 |
| AGI_HUM1_OLIGO_A_24_P861009 | WDR9 ^ WD repeat domain 9 ^ | 0.49 |
| AGI_HUM1_OLIGO_A_23_P257278 | CDK5RAP1 ^ CDK5 regulatory subunit associated protein 1 ^ | 0.75 |
| AGI_HUM1_OLIGO_A_32_P482979 | LOC389677 ^ Similar to RIKEN cDNA 3000004N20 ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P121122 | MAK3 ^ Mak3 homolog (S. cerevisiae) ^ | 0.67 |
| AGI_HUM1_OLIGO_A_23_P169470 | CDK9 ^ Cyclin-dependent kinase 9 (CDC2-related kinase) ^ | 0.70 |
| AGI_HUM1_OLIGO_A_24_P143189 | TMSL3 ^ Thymosin-like 3 ^ | 0.20 |
| AGI_HUM1_OLIGO_A_23_P20022 | HIG2 ^ Hypoxia-inducible protein 2 ^ | 0.64 |
| AGI_HUM1_OLIGO_A_24_P127442 | Uncharacterized gene | 0.60 |
| AGI_HUM1_OLIGO_A_23_P97328 | C1orf35 ^ Chromosome 1 open reading frame 35 ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P385771 | PAOX ^ Polyamine oxidase (exo-N4-amino) ^ | 0.47 |
| AGI_HUM1_OLIGO_A_23_P6307 | U2AF1 ^ U2(RNU2) small nuclear RNA auxiliary factor 1 ^ | 0.75 |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P677634 | Uncharacterized gene | 0.73 |
| AGI_HUM1_OLIGO_A_24_P898915 | FLJ45445 ^ FLJ45445 protein ^ | 0.81 |
| AGI_HUM1_OLIGO_A_23_P10559 | AATK ^ Apoptosis-associated tyrosine kinase ^ | 0.59 |
| AGI_HUM1_OLIGO_A_23_P500734 | POGZ ^ Pogo transposable element with ZNF domain ^ | 0.71 |
| AGI_HUM1_OLIGO_A_23_P77493 | TUBB3 ^ Tubulin, beta 3 ^ | 0.50 |
| AGI_HUM1_OLIGO_A_23_P152115 | NME3 ^ Non-metastatic cells 3, protein expressed in ^ | 0.79 |
| AGI_HUM1_OLIGO_A_23_P717 | FLJ10874 ^ Hypothetical protein FLJ10874 ^ | 0.49 |
| AGI_HUM1_OLIGO_A_24_P182433 | MAPBPIP ^ Mitogen-activated protein-binding protein-interacting protein ^ | 0.61 |
| AGI_HUM1_OLIGO_A_23_P201731 | TRAF5 ^ TNF receptor-associated factor 5 ^ | 0.16 |
| AGI_HUM1_OLIGO_A_23_P60591 | DNAJC7 ^ DnaJ (Hsp40) homolog, subfamily C, member 7 ^ | 0.43 |
| AGI_HUM1_OLIGO_A_23_P307940 | CAPZA2 ^ Capping protein (actin filament) muscle Z-line, alpha 2 ^ | 0.72 |
| AGI_HUM1_OLIGO_A_32_P167493 | SAP18 ^ Sin3-associated polypeptide, 18kDa ^ | 0.86 |
| AGI_HUM1_OLIGO_A_23_P32175 | LHX6 ^ LIM homeobox 6 ^ | 0.35 |
| AGI_HUM1_OLIGO_A_23_P29836 | MGC29956 ^ Hypothetical protein MGC29956 ^ | 0.36 |
| AGI_HUM1_OLIGO_A_32_P158302 | Uncharacterized gene | 0.63 |
| AGI_HUM1_OLIGO_A_23_P161719 | CWF19L2 ^ CWF19-like 2, cell cycle control (S. pombe) ^ | 0.58 |
| AGI_HUM1_OLIGO_A_23_P28969 | C20orf178 ^ Chromosome 20 open reading frame 178 ^ | 0.29 |
| AGI_HUM1_OLIGO_A_23_P55376 | LOC284058 ^ Hypothetical protein LOC284058 ^ | 0.56 |
| AGI_HUM1_OLIGO_A_23_P124855 | ZCCHC7 ^ Zinc finger, CCHC domain containing 7 ^ | 0.52 |
| AGI_HUM1_OLIGO_A_23_P37088 | RDH12 ^ Retinol dehydrogenase 12 (all-trans and 9-cis) ^ | 0.61 |
| AGI_HUM1_OLIGO_A_23_P102391 | SLC40A1 ^ Solute carrier family 40 (iron-regulated transporter), member 1 ^ | 0.26 |
| AGI_HUM1_OLIGO_A_24_P913431 | TRIO ^ Triple functional domain (PTPRF interacting) ^ | 0.54 |
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| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P500421 | EYA2 ^ Eyes absent homolog 2 (Drosophila) ^ | 0.09 |
| AGI_HUM1_OLIGO_A_24_P154214 | IRF2BP2 ^ Interferon regulatory factor 2 binding protein 2 ^ | 0.48 |
| AGI_HUM1_OLIGO_A_23_P40156 | C20orf121 ^ Chromosome 20 open reading frame 121 ^ | 0.54 |
| AGI_HUM1_OLIGO_A_24_P209204 | C6orf62 ^ Chromosome 6 open reading frame 62 ^ | 0.40 |
| AGI_HUM1_OLIGO_A_23_P51548 | MGST3 ^ Microsomal glutathione S-transferase 3 ^ | 0.58 |
| AGI_HUM1_OLIGO_A_23_P22625 | SLC9A6 ^ Solute carrier family 9 (sodium/hydrogen exchanger), isoform 6 ^ | 0.67 |
| AGI_HUM1_OLIGO_A_24_P124672 | DNCL1 ^ Dynein, cytoplasmic, light polypeptide 1 ^ | 0.86 |
| AGI_HUM1_OLIGO_A_24_P254346 | DSCR3 ^ Down syndrome critical region gene 3 ^ | 0.66 |
| AGI_HUM1_OLIGO_A_23_P149847 | LZTS2 ^ Leucine zipper, putative tumor suppressor 2 ^ | 0.78 |
| AGI_HUM1_OLIGO_A_24_P90097 | ADD3 ^ Adducin 3 (gamma) ^ | 0.69 |
| AGI_HUM1_OLIGO_A_23_P123874 | SMU1 ^ Smu-1 suppressor of mec-8 and unc-52 homolog (C. elegans) ^ | 0.58 |
| AGI_HUM1_OLIGO_A_23_P164826 | RNASEH2A ^ Ribonuclease H2, large subunit ^ | 0.87 |
| AGI_HUM1_OLIGO_A_23_P99253 | LIN7A ^ Lin-7 homolog A (C. elegans) ^ | 0.40 |
| AGI_HUM1_OLIGO_A_23_P418413 | OXSR1 ^ Oxidative-stress responsive 1 ^ | 0.51 |
| AGI_HUM1_OLIGO_A_23_P52127 | ACBD6 ^ Acyl-Coenzyme A binding domain containing 6 ^ | 0.59 |
| AGI_HUM1_OLIGO_A_24_P111912 | DKFZP564D172 ^ Hypothetical protein FLJ12078 ^ | 0.64 |
| AGI_HUM1_OLIGO_A_23_P48676 | PYGL ^ Phosphorylase, glycogen; liver (Hers disease, glycogen storage disease type VI) ^ | 0.53 |
| AGI_HUM1_OLIGO_A_24_P366457 | Uncharacterized gene | 0.86 |
| AGI_HUM1_OLIGO_A_23_P12503 | NUP133 ^ Nucleoporin 133kDa ^ | 0.40 |
| AGI_HUM1_OLIGO_A_23_P11874 | MPZL1 ^ Myelin protein zero-like 1 ^ | 0.66 |
| AGI_HUM1_OLIGO_A_23_P88522 | NMB ^ Neuromedin B ^ | 0.29 |
| AGI_HUM1_OLIGO_A_23_P73801 | TCEAL1 ^ Transcription elongation factor A (SII)-like 1 ^ | 0.68 |
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| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P372874 | Uncharacterized gene | 0.43 |
| AGI_HUM1_OLIGO_A_23_P15123 | KIAA1970 ^ KIAA1970 protein ^ | 0.81 |
| AGI_HUM1_OLIGO_A_24_P917866 | SET ^ SET translocation (myeloid leukemia-associated) ^ | 0.68 |
| AGI_HUM1_OLIGO_A_24_P230877 | ^ CDNA FLJ33330 fis, clone BRACE2000441 ^ | 0.79 |
| AGI_HUM1_OLIGO_A_24_P137434 | DCBLD2 ^ Discoidin, CUB and LCCL domain containing 2 ^ | 0.41 |
| AGI_HUM1_OLIGO_A_23_P97749 | DHX9 ^ DEAH (Asp-Glu-Ala-His) box polypeptide 9 ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P257296 | TFF3 ^ Trefoil factor 3 (intestinal) ^ | 0.50 |
| AGI_HUM1_OLIGO_A_23_P38167 | GPRC5C ^ G protein-coupled receptor, family C, group 5, member C ^ | 0.40 |
| AGI_HUM1_OLIGO_A_23_P319859 | EYA2 ^ Eyes absent homolog 2 (Drosophila) ^ | 0.19 |
| AGI_HUM1_OLIGO_A_24_P293192 | FXYD3 ^ FXYD domain containing ion transport regulator 3 ^ | 0.54 |
| AGI_HUM1_OLIGO_A_24_P340286 | LOC150356 ^ Hypothetical protein BC012882 ^ | 0.12 |
| AGI_HUM1_OLIGO_A_24_P200603 | DCOHM ^ Dimerization cofactor of hepatocyte nuclear factor 1 ( HNF1) from muscle ^ | 0.90 |
| AGI_HUM1_OLIGO_A_23_P72737 | IFITM1 ^ Interferon induced transmembrane protein 1 (9-27) ^ | 0.42 |
| AGI_HUM1_OLIGO_A_23_P91487 | C21orf6 ^ Chromosome 21 open reading frame 6 ^ | 0.48 |
| AGI_HUM1_OLIGO_A_23_P371824 | TUFT1 ^ Tuftelin 1 ^ | 0.51 |
| AGI_HUM1_OLIGO_A_24_P141332 | CAMK2G ^ Calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma ^ | 0.76 |
| AGI_HUM1_OLIGO_A_23_P349882 | PDCL ^ Phosducin-like ^ | 0.64 |
| AGI_HUM1_OLIGO_A_23_P109345 | PTTG1IP ^ Pituitary tumor-transforming 1 interacting protein ^ | 0.75 |
| AGI_HUM1_OLIGO_A_23_P359111 | PAI-RBP1 ^ PAI-1 mRNA-binding protein ^ | 0.79 |
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| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P428849 | CHCHD4 ^ Coiled-coil-helix-coiled-coil-helix domain containing 4 ^ | 0.86 |
| AGI_HUM1_OLIGO_A_24_P9671 | DNAJA1 ^ DnaJ (Hsp40) homolog, subfamily A, member 1 ^ | 0.93 |
| AGI_HUM1_OLIGO_A_23_P59798 | MKRN1 ^ Makorin, ring finger protein, 1 ^ | 0.86 |
| AGI_HUM1_OLIGO_A_32_P182473 | ZNF625 ^ Zinc finger protein 625 ^ | 0.72 |
| AGI_HUM1_OLIGO_A_23_P109974 | RAB6B ^ RAB6B, member RAS oncogene family ^ | 0.58 |
| AGI_HUM1_OLIGO_A_23_P138725 | MARVELD1 ^ MARVEL domain containing 1 ^ | 0.48 |
| AGI_HUM1_OLIGO_A_32_P223859 | SMPDL3A ^ Sphingomyelin phosphodiesterase, acid-like 3A ^ | 0.65 |
| AGI_HUM1_OLIGO_A_23_P433107 | ZNF561 ^ Zinc finger protein 561 ^ | 0.45 |
| AGI_HUM1_OLIGO_A_24_P216765 | TOMM20 ^ Translocase of outer mitochondrial membrane 20 homolog (yeast) ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P37560 | PEX11A ^ Peroxisomal biogenesis factor 11A ^ | 0.78 |
| AGI_HUM1_OLIGO_A_23_P211899 | Uncharacterized gene | 0.39 |
| AGI_HUM1_OLIGO_A_23_P150255 | RBM14 ^ RNA binding motif protein 14 ^ | 0.78 |
| AGI_HUM1_OLIGO_A_32_P12820 | Uncharacterized gene | 0.45 |
| AGI_HUM1_OLIGO_A_23_P170037 | MID1 ^ Midline 1 (Opitz/BBB syndrome) ^ | 0.30 |
| AGI_HUM1_OLIGO_A_23_P62920 | KIFAP3 ^ Kinesin-associated protein 3 ^ | 0.65 |
| AGI_HUM1_OLIGO_A_23_P257201 | RNF146 ^ Ring finger protein 146 ^ | 0.79 |
| AGI_HUM1_OLIGO_A_24_P200652 | C6orf62 ^ Chromosome 6 open reading frame 62 ^ | 0.29 |
| AGI_HUM1_OLIGO_A_23_P137514 | IVNS1ABP ^ Influenza virus NS1A binding protein ^ | 0.47 |
| AGI_HUM1_OLIGO_A_24_P55250 | C6orf74 ^ Chromosome 6 open reading frame 74 ^ | 0.86 |
| AGI_HUM1_OLIGO_A_23_P435407 | GPC4 ^ Glypican 4 ^ | 0.56 |
| AGI_HUM1_OLIGO_A_23_P251248 | TBCC ^ Tubulin-specific chaperone c ^ | 0.64 |
| AGI_HUM1_OLIGO_A_24_P48204 | SECTM1 ^ Secreted and transmembrane 1 ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P9416 | ACO1 ^ Aconitase 1, soluble ^ | 0.72 |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P867201 | ^ Homo sapiens, clone IMAGE:5277945, mRNA ^ | 0.32 |
| AGI_HUM1_OLIGO_A_23_P31073 | MYB ^ V-myb myeloblastosis viral oncogene homolog (avian) ^ | 0.39 |
| AGI_HUM1_OLIGO_A_24_P530977 | Uncharacterized gene | 0.17 |
| AGI_HUM1_OLIGO_A_32_P3214 | Uncharacterized gene | 0.29 |
| AGI_HUM1_OLIGO_A_32_P19101 | Uncharacterized gene | 0.48 |
| AGI_HUM1_OLIGO_A_24_P605190 | Uncharacterized gene | 0.41 |
| AGI_HUM1_OLIGO_A_23_P27613 | MAN2B1 ^ Mannosidase, alpha, class 2B, member 1 ^ | 0.77 |
| AGI_HUM1_OLIGO_A_24_P548415 | Uncharacterized gene | 0.56 |
| AGI_HUM1_OLIGO_A_23_P200216 | MAGOH ^ Mago-nashi homolog, proliferation-associated (Drosophila) ^ | 0.65 |
| AGI_HUM1_OLIGO_A_23_P59613 | FZD9 ^ Frizzled homolog 9 (Drosophila) ^ | 0.53 |
| AGI_HUM1_OLIGO_A_23_P168306 | WASF1 ^ WAS protein family, member 1 ^ | 0.36 |
| AGI_HUM1_OLIGO_A_23_P121234 | Uncharacterized gene | 0.59 |
| AGI_HUM1_OLIGO_A_32_P133670 | ANP32A ^ Acidic (leucine-rich) nuclear phosphoprotein 32 family, member A ^ | 0.64 |
| AGI_HUM1_OLIGO_A_24_P240187 | LRRN1 ^ Leucine rich repeat neuronal 1 ^ | 0.46 |
| AGI_HUM1_OLIGO_A_23_P256375 | STX4A ^ Syntaxin 4A (placental) ^ | 0.79 |
| AGI_HUM1_OLIGO_A_23_P203120 | IGSF4 ^ Immunoglobulin superfamily, member 4 ^ | 0.32 |

**Table 5.6. The list of the genes that are significantly up regulated in TR-AR-Y267F compared to vector control**

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P928052 | NRP1 ^ Neuropilin 1 ^ | 3.81 |
| AGI_HUM1_OLIGO_A_24_P382591 | Uncharacterized gene | 1.42 |
| AGI_HUM1_OLIGO_A_23_P252413 | Uncharacterized gene | 1.90 |
| AGI_HUM1_OLIGO_A_23_P79911 | PSMF1 ^ Proteasome (prosome, macropain) inhibitor subunit 1 (PI31) ^ | 1.24 |
| AGI_HUM1_OLIGO_A_23_P121657 | HS3ST1 ^ Heparan sulfate (glucosamine) 3-O-sulfotransferase 1 ^ | 2.06 |
| AGI_HUM1_OLIGO_A_23_P54540 | Uncharacterized gene | 1.52 |
| AGI_HUM1_OLIGO_A_32_P51518 | ^ CDNA FLJ40901 fis, clone UTERU2003704 ^ | 1.55 |
| AGI_HUM1_OLIGO_A_23_P90679 | ALS2CR2 ^ Amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 2 ^ | 1.54 |
| AGI_HUM1_OLIGO_A_23_P428260 | STEAP2 ^ Six transmembrane epithelial antigen of prostate 2 ^ | 2.28 |
| AGI_HUM1_OLIGO_A_24_P658427 | NFIB ^ Nuclear factor I/B ^ | 1.90 |
| AGI_HUM1_OLIGO_A_23_P382043 | NT5C2L1 ^ 5'-nucleotidase, cytosolic II-like 1 ^ | 1.64 |
| AGI_HUM1_OLIGO_A_23_P372234 | CA12 ^ Carbonic anhydrase XII ^ | 2.93 |
| AGI_HUM1_OLIGO_A_23_P145644 | DDC ^ Dopa decarboxylase (aromatic L-amino acid decarboxylase) ^ | 6.70 |
| AGI_HUM1_OLIGO_A_23_P123330 | RPL30 ^ Ribosomal protein L30 ^ | 1.33 |
| AGI_HUM1_OLIGO_A_24_P48898 | APOL2 ^ Apolipoprotein L, 2 ^ | 1.35 |
| AGI_HUM1_OLIGO_A_24_P271285 | BTBD14B ^ BTB (POZ) domain containing 14B ^ | 1.31 |
| AGI_HUM1_OLIGO_A_23_P310274 | PRSS2 ^ Protease, serine, 2 (trypsin 2) ^ | 8.47 |
| AGI_HUM1_OLIGO_A_23_P15889 | CBLN2 ^ Cerebellin 2 precursor ^ | 5.90 |
| AGI_HUM1_OLIGO_A_23_P2223 | MLC1SA ^ Myosin light chain 1 slow a ^ | 1.10 |
| AGI_HUM1_OLIGO_A_23_P91699 | EIF3S7 ^ Eukaryotic translation initiation factor 3, subunit 7 zeta, 66/67kDa ^ | 1.32 |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P67976 | ZNF403 ^ Zinc finger protein 403 ^ | 1.60 |
| AGI_HUM1_OLIGO_A_24_P918808 | OAZ1 ^ Ornithine decarboxylase antizyme 1 ^ | 1.87 |
| AGI_HUM1_OLIGO_A_24_P221869 | TSPY1 ^ Testis specific protein, Y-linked 1 ^ | 4.28 |
| AGI_HUM1_OLIGO_A_23_P141208 | DKFZp566O084 ^ DKFZP566O084 protein ^ | 1.34 |
| AGI_HUM1_OLIGO_A_24_P298886 | Uncharacterized gene | 3.63 |
| AGI_HUM1_OLIGO_A_32_P165477 | SLC7A11 ^ Solute carrier family 7, (cationic amino acid transporter, y+ system) member 11 ^ | 1.87 |
| AGI_HUM1_OLIGO_A_23_P132910 | FLJ20273 ^ RNA-binding protein ^ | 1.50 |
| AGI_HUM1_OLIGO_A_23_P138706 | ADRA2A ^ Adrenergic, alpha-2A-, receptor ^ | 5.06 |
| AGI_HUM1_OLIGO_A_23_P218170 | SMAP-1 ^ Smooth muscle cell associated protein-1 ^ | 1.36 |
| AGI_HUM1_OLIGO_A_23_P83953 | Uncharacterized gene | 1.32 |
| AGI_HUM1_OLIGO_A_23_P99967 | BBS4 ^ Bardet-Biedl syndrome 4 ^ | 2.36 |
| AGI_HUM1_OLIGO_A_23_P91619 | MIF ^ Macrophage migration inhibitory factor (glycosylation-inhibiting factor) ^ | 1.78 |
| AGI_HUM1_OLIGO_A_23_P140450 | SLC27A2 ^ Solute carrier family 27 (fatty acid transporter), member 2 ^ | 1.82 |
| AGI_HUM1_OLIGO_A_23_P3632 | ANKRD11 ^ Ankyrin repeat domain 11 ^ | 2.08 |
| AGI_HUM1_OLIGO_A_24_P409891 | Uncharacterized gene | 1.24 |
| AGI_HUM1_OLIGO_A_23_P22800 | TSPY1 ^ Testis specific protein, Y-linked 1 ^ | 2.89 |
| AGI_HUM1_OLIGO_A_23_P117928 | BAHD1 ^ Bromo adjacent homology domain containing 1 ^ | 1.47 |
| AGI_HUM1_OLIGO_A_23_P110686 | STC2 ^ Stanniocalcin 2 ^ | 1.50 |
| AGI_HUM1_OLIGO_A_23_P213102 | KIAA0992 ^ Palladin ^ | 1.54 |
| AGI_HUM1_OLIGO_A_24_P101201 | GRP58 ^ Glucose regulated protein, 58kDa ^ | 1.53 |
| AGI_HUM1_OLIGO_A_24_P881430 | ^ Transcribed locus, moderately similar to NP_000991.1 ribosomal protein L39 [Homo sapiens] ^ | 2.12 |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P246552 | VTI1B ^ Vesicle transport through interaction with t-SNAREs homolog 1B (yeast) ^ | 1.63 |
| AGI_HUM1_OLIGO_A_23_P37983 | MT1B ^ Metallothionein 1B (functional) ^ | 2.69 |
| AGI_HUM1_OLIGO_A_23_P375147 | Uncharacterized gene | 1.49 |
| AGI_HUM1_OLIGO_A_24_P203726 | Uncharacterized gene | 1.37 |
| AGI_HUM1_OLIGO_A_23_P56150 | MGC15631 ^ Hypothetical protein MGC15631 ^ | 1.14 |
| AGI_HUM1_OLIGO_A_24_P84048 | Uncharacterized gene | 1.10 |
| AGI_HUM1_OLIGO_A_23_P381102 | DKFZp434E2321 ^ Hypothetical protein DKFZp434E2321 ^ | 1.85 |
| AGI_HUM1_OLIGO_A_23_P321949 | PLA2G2A ^ Phospholipase A2, group IIA (platelets, synovial fluid) ^ | 4.47 |
| AGI_HUM1_OLIGO_A_32_P139414 | FEM1A ^ Fem-1 homolog a (C.elegans) ^ | 2.15 |
| AGI_HUM1_OLIGO_A_23_P409945 | OAZ1 ^ Ornithine decarboxylase antizyme 1 ^ | 1.45 |
| AGI_HUM1_OLIGO_A_23_P402287 | LNX2 ^ Ligand of numb-protein X 2 ^ | 1.56 |
| AGI_HUM1_OLIGO_A_24_P44462 | TPM1 ^ Tropomyosin 1 (alpha) ^ | 1.49 |
| AGI_HUM1_OLIGO_A_24_P363802 | PSMD5 ^ Hypothetical protein LOC253039 ^ | 4.88 |
| AGI_HUM1_OLIGO_A_23_P214766 | HIVEP2 ^ Human immunodeficiency virus type I enhancer binding protein 2 ^ | 1.69 |
| AGI_HUM1_OLIGO_A_23_P88347 | PLEKHC1 ^ Pleckstrin homology domain containing, family C (with FERM domain) member 1 ^ | 2.30 |
| AGI_HUM1_OLIGO_A_23_P119943 | IGFBP2 ^ Insulin-like growth factor binding protein 2, 36kDa ^ | 1.05 |
| AGI_HUM1_OLIGO_A_23_P108244 | COX6B1 ^ Cytochrome c oxidase subunit Vib polypeptide 1 (ubiquitous) ^ | 1.34 |
| AGI_HUM1_OLIGO_A_23_P15542 | HSD17B1 ^ Hydroxysteroid (17-beta) dehydrogenase 1 ^ | 1.46 |
| AGI_HUM1_OLIGO_A_24_P116242 | KLHDC2 ^ Kelch domain containing 2 ^ | 1.19 |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P263672 | LOC96610 ^ Hypothetical protein similar to KIAA0187 gene product ^ | 1.55 |
| AGI_HUM1_OLIGO_A_23_P121356 | BBX ^ Bobby sox homolog (Drosophila) ^ | 2.47 |
| AGI_HUM1_OLIGO_A_23_P66719 | MGC23280 ^ Hypothetical protein MGC23280 ^ | 1.46 |
| AGI_HUM1_OLIGO_A_32_P71736 | PIGH ^ Phosphatidylinositol glycan, class H ^ | 1.42 |
| AGI_HUM1_OLIGO_A_23_P29855 | VDP ^ Vesicle docking protein p115 ^ | 1.42 |
| AGI_HUM1_OLIGO_A_23_P125519 | RPS4X ^ Ribosomal protein S4, X-linked ^ | 1.15 |
| AGI_HUM1_OLIGO_A_24_P363615 | MTPN ^ Myotrophin ^ | 1.84 |
| AGI_HUM1_OLIGO_A_24_P67892 | ZNF403 ^ Zinc finger protein 403 ^ | 1.55 |
| AGI_HUM1_OLIGO_A_23_P139958 | CDK8 ^ Cyclin-dependent kinase 8 ^ | 1.97 |
| AGI_HUM1_OLIGO_A_23_P93613 | MKLN1 ^ Muskelin 1, intracellular mediator containing kelch motifs ^ | 1.57 |
| AGI_HUM1_OLIGO_A_23_P99883 | GRP58 ^ Glucose regulated protein, 58kDa ^ | 1.60 |
| AGI_HUM1_OLIGO_A_23_P25204 | SLC25A3 ^ Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3 ^ | 1.17 |

**Table 5.7. The list of the genes that are significantly down regulated in TR-AR-Y267F compared to vector control**

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P114783 | PARP1 ^ Poly (ADP-ribose) polymerase family, member 1 ^ | 0.43 |
| AGI_HUM1_OLIGO_A_23_P216257 | TPD52 ^ Tumor protein D52 ^ | 0.67 |
| AGI_HUM1_OLIGO_A_23_P150255 | RBM14 ^ RNA binding motif protein 14 ^ | 0.62 |
| AGI_HUM1_OLIGO_A_32_P119830 | PEG3 ^ Paternally expressed 3 ^ | 0.16 |
| AGI_HUM1_OLIGO_A_24_P185394 | GSK3A ^ Glycogen synthase kinase 3 alpha ^ | 0.81 |
| AGI_HUM1_OLIGO_A_23_P49517 | SFRS1 ^ Splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor) ^ | 0.70 |
| AGI_HUM1_OLIGO_A_23_P148519 | TMEM29 ^ Transmembrane protein 29 ^ | 0.80 |
| AGI_HUM1_OLIGO_A_24_P307486 | Uncharacterized gene | 0.68 |
| AGI_HUM1_OLIGO_A_23_P171077 | EBP ^ Emopamil binding protein (sterol isomerase) ^ | 0.62 |
| AGI_HUM1_OLIGO_A_24_P61864 | GK001 ^ GK001 protein ^ | 0.55 |
| AGI_HUM1_OLIGO_A_32_P96036 | LOC92312 ^ Hypothetical protein LOC92312 ^ | 0.34 |
| AGI_HUM1_OLIGO_A_23_P104651 | CDCA5 ^ Cell division cycle associated 5 ^ | 0.50 |
| AGI_HUM1_OLIGO_A_23_P70060 | PPAP2A ^ Phosphatidic acid phosphatase type 2A ^ | 0.64 |
| AGI_HUM1_OLIGO_A_24_P366495 | Uncharacterized gene | 0.58 |
| AGI_HUM1_OLIGO_A_24_P857404 | LOC92312 ^ Hypothetical protein LOC92312 ^ | 0.40 |
| AGI_HUM1_OLIGO_A_23_P103414 | YTHDF2 ^ YTH domain family, member 2 ^ | 0.60 |
| AGI_HUM1_OLIGO_A_23_P251795 | GPC2 ^ Glypican 2 (cerebroglycan) ^ | 0.63 |
| AGI_HUM1_OLIGO_A_23_P200477 | ACBD3 ^ Acyl-Coenzyme A binding domain containing 3 ^ | 0.55 |
| AGI_HUM1_OLIGO_A_24_P382119 | MTMR4 ^ Myotubularin related protein 4 ^ | 0.71 |
| AGI_HUM1_OLIGO_A_23_P17074 | MGC12981 ^ Hypothetical protein MGC12981 ^ | 0.75 |
| AGI_HUM1_OLIGO_A_32_P11181 | Uncharacterized gene | 0.74 |
| AGI_HUM1_OLIGO_A_23_P122304 | HDAC2 ^ Histone deacetylase 2 ^ | 0.70 |
| AGI_HUM1_OLIGO_A_23_P200710 | PIK3C2B ^ Phosphoinositide-3-kinase, class 2, beta polypeptide ^ | 0.84 |
| | | |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P76901 | PLEKHG3 ^ Pleckstrin homology domain containing, family G (with RhoGef domain) member 3 ^ | 0.70 |
| AGI_HUM1_OLIGO_A_23_P103398 | PSEN2 ^ Presenilin 2 (Alzheimer disease 4) ^ | 0.69 |
| AGI_HUM1_OLIGO_A_23_P160406 | KCTD3 ^ Potassium channel tetramerisation domain containing 3 ^ | 0.67 |
| AGI_HUM1_OLIGO_A_24_P387839 | DEGS ^ Degenerative spermatocyte homolog, lipid desaturase (Drosophila) ^ | 0.76 |
| AGI_HUM1_OLIGO_A_23_P218456 | ILF3 ^ Interleukin enhancer binding factor 3, 90kDa ^ | 0.80 |
| AGI_HUM1_OLIGO_A_23_P143981 | FBLN2 ^ Fibulin 2 ^ | 0.39 |
| AGI_HUM1_OLIGO_A_23_P161190 | VIM ^ Vimentin ^ | 0.15 |
| AGI_HUM1_OLIGO_A_23_P49546 | GRIN2C ^ Glutamate receptor, ionotropic, N-methyl D-aspartate 2C ^ | 0.23 |
| AGI_HUM1_OLIGO_A_23_P107421 | TK1 ^ Thymidine kinase 1, soluble ^ | 0.50 |
| AGI_HUM1_OLIGO_A_23_P200901 | ENSA ^ Endosulfine alpha ^ | 0.56 |
| AGI_HUM1_OLIGO_A_23_P343382 | Uncharacterized gene | 0.24 |
| AGI_HUM1_OLIGO_A_24_P123601 | DDR1 ^ Discoidin domain receptor family, member 1 ^ | 0.66 |
| AGI_HUM1_OLIGO_A_23_P411833 | LOC197336 ^ Similar to RIKEN cDNA 3230401M21 [Mus musculus] ^ | 0.49 |
| AGI_HUM1_OLIGO_A_24_P682601 | Uncharacterized gene | 0.47 |
| AGI_HUM1_OLIGO_A_23_P50775 | LRFN3 ^ Leucine rich repeat and fibronectin type III domain containing 3 ^ | 0.65 |
| AGI_HUM1_OLIGO_A_32_P191895 | ^ Homo sapiens, Similar to syndecan binding protein (syntenin), clone IMAGE:4814292, mRNA ^ | 0.48 |
| AGI_HUM1_OLIGO_A_23_P45917 | CKS1B ^ CDC28 protein kinase regulatory subunit 1B ^ | 0.52 |
| AGI_HUM1_OLIGO_A_24_P414419 | HADHSC ^ L-3-hydroxyacyl-Coenzyme A dehydrogenase, short chain ^ | 0.65 |
| AGI_HUM1_OLIGO_A_23_P39364 | HOMER3 ^ Homer homolog 3 (Drosophila) ^ | 0.54 |
| AGI_HUM1_OLIGO_A_32_P12183 | LOC349114 ^ Hypothetical protein LOC349114 ^ | 0.63 |
| AGI_HUM1_OLIGO_A_32_P456537 | TCP1 ^ T-complex 1 ^ | 0.68 |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_32_P146635 | SESN3 ^ Sestrin 3 ^ | 0.54 |
| AGI_HUM1_OLIGO_A_23_P428849 | CHCHD4 ^ Coiled-coil-helix-coiled-coil-helix domain containing 4 ^ | 0.83 |
| AGI_HUM1_OLIGO_A_23_P120153 | RNF149 ^ Ring finger protein 149 ^ | 0.46 |
| AGI_HUM1_OLIGO_A_23_P214678 | C6orf82 ^ Chromosome 6 open reading frame 82 ^ | 0.79 |
| AGI_HUM1_OLIGO_A_23_P421423 | TNFAIP2 ^ Tumor necrosis factor, alpha-induced protein 2 ^ | 0.27 |
| AGI_HUM1_OLIGO_A_23_P130027 | EPN3 ^ Epsin 3 ^ | 0.67 |
| AGI_HUM1_OLIGO_A_23_P110941 | GSTA4 ^ Glutathione S-transferase A4 ^ | 0.66 |
| AGI_HUM1_OLIGO_A_23_P77360 | CIAO1 ^ WD40 protein Ciao1 ^ | 0.80 |
| AGI_HUM1_OLIGO_A_23_P59791 | LUC7L2 ^ LUC7-like 2 (S. cerevisiae) ^ | 0.53 |
| AGI_HUM1_OLIGO_A_23_P99249 | SUCLG2 ^ Succinate-CoA ligase, GDP-forming, beta subunit ^ | 0.60 |
| AGI_HUM1_OLIGO_A_23_P104372 | DNAJC9 ^ DnaJ (Hsp40) homolog, subfamily C, member 9 ^ | 0.69 |
| AGI_HUM1_OLIGO_A_23_P70409 | POLR1C ^ Polymerase (RNA) I polypeptide C, 30kDa ^ | 0.70 |
| AGI_HUM1_OLIGO_A_23_P46507 | CGI-143 ^ CGI-143 protein ^ | 0.57 |
| AGI_HUM1_OLIGO_A_23_P12874 | GTPBP4 ^ GTP binding protein 4 ^ | 0.76 |
| AGI_HUM1_OLIGO_A_24_P314337 | GAGEB1 ^ G antigen, family B, 1 (prostate associated) ^ | 0.26 |
| AGI_HUM1_OLIGO_A_23_P94422 | MELK ^ Maternal embryonic leucine zipper kinase ^ | 0.67 |
| AGI_HUM1_OLIGO_A_23_P110837 | IRX4 ^ Iroquois homeobox protein 4 ^ | 0.45 |
| AGI_HUM1_OLIGO_A_23_P90612 | MCM6 ^ MCM6 minichromosome maintenance deficient 6 (MIS5 homolog, S. pombe) (S. cerevisiae) ^ | 0.66 |
| AGI_HUM1_OLIGO_A_24_P229616 | Uncharacterized gene | 0.79 |
| AGI_HUM1_OLIGO_A_24_P339272 | MGC40157 ^ Hypothetical protein MGC40157 ^ | 0.82 |
| AGI_HUM1_OLIGO_A_32_P114574 | CACYBP ^ Calcyclin binding protein ^ | 0.55 |
| AGI_HUM1_OLIGO_A_23_P319859 | EYA2 ^ Eyes absent homolog 2 (Drosophila) ^ | 0.17 |
| AGI_HUM1_OLIGO_A_23_P43946 | CIP29 ^ Cytokine induced protein 29 kDa ^ | 0.49 |
| AGI_HUM1_OLIGO_A_32_P31633 | SF3B1 ^ Splicing factor 3b, subunit 1, 155kDa ^ | 0.87 |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P839239 | Uncharacterized gene | 0.42 |
| AGI_HUM1_OLIGO_A_24_P38895 | H2AFX ^ H2A histone family, member X ^ | 0.64 |
| AGI_HUM1_OLIGO_A_23_P86330 | IER5 ^ Immediate early response 5 ^ | 0.59 |
| AGI_HUM1_OLIGO_A_24_P195831 | Uncharacterized gene | 0.60 |
| AGI_HUM1_OLIGO_A_23_P374844 | GAL ^ Galanin ^ | 0.42 |
| AGI_HUM1_OLIGO_A_23_P51660 | MUTYH ^ MutY homolog (E. coli) ^ | 0.86 |
| AGI_HUM1_OLIGO_A_23_P118462 | DPH2L1 ^ Candidate tumor suppressor in ovarian cancer 2 ^ | 0.79 |
| AGI_HUM1_OLIGO_A_24_P178415 | Uncharacterized gene | 0.71 |
| AGI_HUM1_OLIGO_A_24_P924484 | Uncharacterized gene | 0.47 |
| AGI_HUM1_OLIGO_A_23_P34375 | TCEA3 ^ Transcription elongation factor A (SII), 3 ^ | 0.19 |
| AGI_HUM1_OLIGO_A_24_P343869 | TEBP ^ Unactive progesterone receptor, 23 kD ^ | 0.65 |
| AGI_HUM1_OLIGO_A_23_P256413 | CKLFSF7 ^ Chemokine-like factor super family 7 ^ | 0.70 |
| AGI_HUM1_OLIGO_A_23_P381979 | OGT ^ O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase) ^ | 0.60 |
| AGI_HUM1_OLIGO_A_23_P20502 | SLC39A4 ^ Solute carrier family 39 (zinc transporter), member 4 ^ | 0.76 |
| AGI_HUM1_OLIGO_A_24_P464798 | Uncharacterized gene | 0.66 |
| AGI_HUM1_OLIGO_A_23_P200493 | LBR ^ Lamin B receptor ^ | 0.58 |
| AGI_HUM1_OLIGO_A_24_P228796 | GAGE2 ^ G antigen 7 ^ | 0.52 |
| AGI_HUM1_OLIGO_A_23_P12680 | PSAP ^ Prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy) ^ | 0.59 |
| AGI_HUM1_OLIGO_A_23_P162559 | SPPL3 ^ Signal peptide peptidase 3 ^ | 0.76 |
| AGI_HUM1_OLIGO_A_23_P53276 | TIMELESS ^ Timeless homolog (Drosophila) ^ | 0.46 |
| AGI_HUM1_OLIGO_A_23_P58293 | UBE2D3 ^ Ubiquitin-conjugating enzyme E2D 3 (UBC4/5 homolog, yeast) ^ | 0.58 |
| AGI_HUM1_OLIGO_A_24_P401150 | Uncharacterized gene | 0.85 |
| AGI_HUM1_OLIGO_A_23_P141362 | FZD2 ^ Frizzled homolog 2 (Drosophila) ^ | 0.33 |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P208880 | UHRF1 ^ Ubiquitin-like, containing PHD and RING finger domains, 1 ^ | 0.23 |
| AGI_HUM1_OLIGO_A_23_P116235 | Uncharacterized gene | 0.20 |
| AGI_HUM1_OLIGO_A_23_P82206 | ECHDC1 ^ Enoyl Coenzyme A hydratase domain containing 1 ^ | 0.06 |
| AGI_HUM1_OLIGO_A_23_P58353 | HNRPD ^ Heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa) ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P121095 | TOP2B ^ Topoisomerase (DNA) II beta 180kDa ^ | 0.86 |
| AGI_HUM1_OLIGO_A_23_P56978 | PTK6 ^ PTK6 protein tyrosine kinase 6 ^ | 0.24 |
| AGI_HUM1_OLIGO_A_24_P222684 | SSA2 ^ Sjogren syndrome antigen A2 (60kDa, ribonucleoprotein autoantigen SS-A/Ro) ^ | 0.55 |
| AGI_HUM1_OLIGO_A_23_P68106 | TMSB10 ^ Thymosin, beta 10 ^ | 0.41 |
| AGI_HUM1_OLIGO_A_23_P119562 | DF ^ D component of complement (adipsin) ^ | 0.30 |
| AGI_HUM1_OLIGO_A_23_P256735 | PGCP ^ Plasma glutamate carboxypeptidase ^ | 0.28 |
| AGI_HUM1_OLIGO_A_32_P33576 | OPRK1 ^ Opioid receptor, kappa 1 ^ | 0.39 |
| AGI_HUM1_OLIGO_A_23_P210581 | KCNG1 ^ Potassium voltage-gated channel, subfamily G, member 1 ^ | 0.64 |
| AGI_HUM1_OLIGO_A_23_P254271 | TUBB6 ^ Tubulin, beta 6 ^ | 0.06 |
| AGI_HUM1_OLIGO_A_23_P30813 | HIST1H4K ^ Histone 1, H4k ^ | 0.75 |
| AGI_HUM1_OLIGO_A_23_P97328 | C1orf35 ^ Chromosome 1 open reading frame 35 ^ | 0.52 |
| AGI_HUM1_OLIGO_A_23_P112341 | C9orf76 ^ Chromosome 9 open reading frame 76 ^ | 0.44 |
| AGI_HUM1_OLIGO_A_23_P319270 | Uncharacterized gene | 0.83 |
| AGI_HUM1_OLIGO_A_24_P287785 | CGI-14 ^ CGI-14 protein ^ | 0.70 |
| AGI_HUM1_OLIGO_A_23_P155765 | HMGB2 ^ High-mobility group box 2 ^ | 0.41 |
| AGI_HUM1_OLIGO_A_32_P192430 | CKS1B ^ CDC28 protein kinase regulatory subunit 1B ^ | 0.47 |
| AGI_HUM1_OLIGO_A_32_P24165 | Uncharacterized gene | 0.38 |
| AGI_HUM1_OLIGO_A_23_P74115 | RAD54L ^ RAD54-like (S. cerevisiae) ^ | 0.59 |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| | | |
| AGI_HUM1_OLIGO_A_23_P20722 | SNAPC4 ^ Small nuclear RNA activating complex, polypeptide 4, 190kDa ^ | 0.61 |
| AGI_HUM1_OLIGO_A_32_P52911 | MTCBP-1 ^ Membrane-type 1 matrix metalloproteinase cytoplasmic tail binding protein-1 ^ | 0.75 |
| AGI_HUM1_OLIGO_A_23_P428129 | CDKN1C ^ Cyclin-dependent kinase inhibitor 1C (p57, Kip2) ^ | 0.55 |
| AGI_HUM1_OLIGO_A_24_P791515 | SYNCRIP ^ Synaptotagmin binding, cytoplasmic RNA interacting protein ^ | 0.53 |
| AGI_HUM1_OLIGO_A_23_P50426 | ANKRD25 ^ Ankyrin repeat domain 25 ^ | 0.83 |
| AGI_HUM1_OLIGO_A_24_P244356 | NOD9 ^ NOD9 protein ^ | 0.80 |
| AGI_HUM1_OLIGO_A_23_P66608 | GCN5L2 ^ GCN5 general control of amino-acid synthesis 5-like 2 (yeast) ^ | 0.55 |
| AGI_HUM1_OLIGO_A_23_P61487 | LRRC20 ^ Leucine rich repeat containing 20 ^ | 0.66 |
| AGI_HUM1_OLIGO_A_24_P654066 | Uncharacterized gene | 0.43 |
| AGI_HUM1_OLIGO_A_23_P124522 | FLJ22955 ^ Hypothetical protein FLJ22955 ^ | 0.84 |
| AGI_HUM1_OLIGO_A_23_P341223 | KIAA0469 ^ KIAA0469 gene product ^ | 0.76 |
| AGI_HUM1_OLIGO_A_24_P222022 | CSIG ^ Cellular senescence inhibited gene protein ^ | 0.73 |
| AGI_HUM1_OLIGO_A_23_P212639 | SFRS10 ^ Splicing factor, arginine/serine-rich 10 (transformer 2 homolog, Drosophila) ^ | 0.88 |
| AGI_HUM1_OLIGO_A_24_P280873 | Uncharacterized gene | 0.61 |
| AGI_HUM1_OLIGO_A_23_P309361 | FLJ30525 ^ Hypothetical protein FLJ30525 ^ | 0.02 |
| AGI_HUM1_OLIGO_A_23_P88522 | NMB ^ Neuromedin B ^ | 0.35 |
| AGI_HUM1_OLIGO_A_23_P204484 | RAB35 ^ RAB35, member RAS oncogene family ^ | 0.62 |
| AGI_HUM1_OLIGO_A_24_P15821 | Uncharacterized gene | 0.69 |
| AGI_HUM1_OLIGO_A_23_P142424 | FLJ22573 ^ Hypothetical protein FLJ22573 ^ | 0.67 |
| AGI_HUM1_OLIGO_A_23_P259272 | WSB2 ^ WD repeat and SOCS box-containing 2 ^ | 0.68 |
| AGI_HUM1_OLIGO_A_24_P724040 | SNRPB2 ^ Small nuclear ribonucleoprotein polypeptide B'' ^ | 0.65 |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_23_P65174 | Uncharacterized gene | 0.04 |
| AGI_HUM1_OLIGO_A_24_P229756 | Uncharacterized gene | 0.63 |
| AGI_HUM1_OLIGO_A_32_P173481 | IK ^ IK cytokine, down-regulator of HLA II ^ | 0.82 |
| AGI_HUM1_OLIGO_A_23_P216068 | ATAD2 ^ ATPase family, AAA domain containing 2 ^ | 0.55 |
| AGI_HUM1_OLIGO_A_23_P158725 | SLC16A3 ^ Solute carrier family 16 (monocarboxylic acid transporters), member 3 ^ | 0.18 |
| AGI_HUM1_OLIGO_A_23_P37144 | C14orf126 ^ Chromosome 14 open reading frame 126 ^ | 0.85 |
| AGI_HUM1_OLIGO_A_23_P422026 | ME1 ^ Malic enzyme 1, NADP(+)-dependent, cytosolic ^ | 0.90 |
| AGI_HUM1_OLIGO_A_23_P407583 | PEG3 ^ Paternally expressed 3 ^ | 0.12 |
| AGI_HUM1_OLIGO_A_23_P11674 | PTGFR ^ Prostaglandin F receptor (FP) ^ | 0.09 |
| AGI_HUM1_OLIGO_A_24_P263623 | TEBP ^ Unactive progesterone receptor, 23 kD ^ | 0.75 |
| AGI_HUM1_OLIGO_A_23_P50455 | POLD1 ^ Polymerase (DNA directed), delta 1, catalytic subunit 125kDa ^ | 0.55 |
| AGI_HUM1_OLIGO_A_32_P27020 | MGC8902 ^ Hypothetical protein MGC8902 ^ | 0.60 |
| AGI_HUM1_OLIGO_A_23_P115482 | HSPC150 ^ HSPC150 protein similar to ubiquitin-conjugating enzyme ^ | 0.48 |
| AGI_HUM1_OLIGO_A_23_P110846 | CNOT8 ^ CCR4-NOT transcription complex, subunit 8 ^ | 0.64 |
| AGI_HUM1_OLIGO_A_23_P10385 | RAMP ^ RA-regulated nuclear matrix-associated protein ^ | 0.42 |
| AGI_HUM1_OLIGO_A_23_P34376 | TCEA3 ^ Transcription elongation factor A (SII), 3 ^ | 0.08 |
| AGI_HUM1_OLIGO_A_23_P111171 | B3GALT4 ^ UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 4 ^ | 0.56 |
| AGI_HUM1_OLIGO_A_23_P202696 | KBTBD4 ^ Kelch repeat and BTB (POZ) domain containing 4 ^ | 0.72 |
| AGI_HUM1_OLIGO_A_32_P22263 | HMGN1 ^ High-mobility group nucleosome binding domain 1 ^ | 0.61 |
| AGI_HUM1_OLIGO_A_23_P50096 | TYMS ^ Thymidylate synthetase ^ | 0.79 |
| AGI_HUM1_OLIGO_A_24_P349560 | EIF4E ^ Eukaryotic translation initiation factor 4E ^ | 0.66 |
| AGI_HUM1_OLIGO_A_23_P160546 | FLJ11280 ^ Hypothetical protein FLJ11280 ^ | 0.28 |
| | | |

| Agilent Gene_ID | Gene Name | Fold Change |
|---|---|---|
| AGI_HUM1_OLIGO_A_24_P154214 | IRF2BP2 ^ Interferon regulatory factor 2 binding protein 2 ^ | 0.50 |
| AGI_HUM1_OLIGO_A_24_P4212 | PYCRL ^ Pyrroline-5-carboxylate reductase-like ^ | 0.57 |
| AGI_HUM1_OLIGO_A_23_P400217 | MUM1 ^ Melanoma associated antigen (mutated) 1 ^ | 0.74 |
| AGI_HUM1_OLIGO_A_23_P334751 | MGC39558 ^ Beta 1,3-N-acetylgalactosaminyltransferase-II ^ | 0.64 |
| AGI_HUM1_OLIGO_A_23_P89249 | ERBB2 ^ V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian) ^ | 0.49 |
| AGI_HUM1_OLIGO_A_23_P51627 | FLJ11838 ^ Hypothetical protein FLJ11838 ^ | 0.81 |
| AGI_HUM1_OLIGO_A_32_P11786 | SFRS6 ^ Splicing factor, arginine/serine-rich 6 ^ | 0.33 |
| AGI_HUM1_OLIGO_A_23_P91487 | C21orf6 ^ Chromosome 21 open reading frame 6 ^ | 0.64 |
| AGI_HUM1_OLIGO_A_24_P274795 | RAM2 ^ Transcription factor RAM2 ^ | 0.84 |
| AGI_HUM1_OLIGO_A_23_P24433 | CTSF ^ Cathepsin F ^ | 0.57 |
| AGI_HUM1_OLIGO_A_23_P134650 | PTCD1 ^ Pentatricopeptide repeat domain 1 ^ | 0.79 |
| AGI_HUM1_OLIGO_A_32_P155091 | ATXN2L ^ Ataxin 2-like ^ | 0.70 |
| AGI_HUM1_OLIGO_A_23_P118815 | Uncharacterized gene | 0.67 |
| AGI_HUM1_OLIGO_A_23_P63789 | ZWINT ^ ZW10 interactor ^ | 0.52 |
| AGI_HUM1_OLIGO_A_23_P215419 | ICA1 ^ Islet cell autoantigen 1, 69kDa ^ | 0.83 |
| AGI_HUM1_OLIGO_A_32_P8251 | KIAA1542 ^ CTD-binding SR-like protein rA9 ^ | 0.73 |
| AGI_HUM1_OLIGO_A_23_P11705 | FLJ10276 ^ Hypothetical protein FLJ10276 ^ | 0.83 |
| AGI_HUM1_OLIGO_A_23_P257296 | TFF3 ^ Trefoil factor 3 (intestinal) ^ | 0.62 |
| AGI_HUM1_OLIGO_A_23_P391506 | IVNS1ABP ^ Influenza virus NS1A binding protein ^ | 0.36 |

**Table 5.8. Intra class correlation (ICC) matrix of microarray samples.**

| | FL-WT-AR-1 | FL-WT-AR-2 | FL-AR-Y267F-1 | FL-AR-Y267F-2 | TR-WT-AR-1 | TR-WT-AR-2 | TR-AR-Y267F-1 | TR-AR-Y267F-2 |
|---|---|---|---|---|---|---|---|---|
| **FL-WT-AR-1** | **1** | **0.92** | 0.57 | 0.63 | 0.89 | 0.83 | 0.79 | 0.56 |
| **FL-WT-AR-2** | | **1** | 0.60 | 0.68 | 0.83 | 0.84 | 0.84 | 0.62 |
| **FL-AR-Y267F-1** | | | **1** | **0.78** | 0.49 | 0.45 | 0.55 | 0.31 |
| **FL-AR-Y267F-2** | | | | **1** | 0.57 | 0.68 | 0.70 | 0.32 |
| **TR-WT-AR-1** | | | | | **1** | **0.88** | 0.82 | 0.66 |
| **TR-WT-AR-2** | | | | | | **1** | 0.89 | 0.57 |
| **TR-AR-Y267F-1** | | | | | | | **1** | **0.74** |
| **TR-AR-Y267F-2** | | | | | | | | **1** |