

Award Number: W81XWH-11-1-0204

TITLE: MicroRNA Targets of Human Androgen Receptor

PRINCIPAL INVESTIGATOR: Girish C Shukla

CONTRACTING ORGANIZATION: Cleveland State University
Cleveland, OH 44115-2214

REPORT DATE: May 2013

TYPE OF REPORT: Final Addendum

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE May 2013			2. REPORT TYPE Final Addendum			3. DATES COVERED 1 May 2012- 30 April 2013			
4. TITLE AND SUBTITLE MicroRNA Targets of Human Androgen Receptor						5a. CONTRACT NUMBER			
						5b. GRANT NUMBER W81XWH-11-1-0204			
						5c. PROGRAM ELEMENT NUMBER			
6. AUTHOR(S) Girish C Shukla						5d. PROJECT NUMBER			
						5e. TASK NUMBER			
						5f. WORK UNIT NUMBER			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Cleveland State University Cleveland, OH 44115-2214						8. PERFORMING ORGANIZATION REPORT NUMBER			
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012						10. SPONSOR/MONITOR'S ACRONYM(S)			
						11. SPONSOR/MONITOR'S REPORT NUMBER(S)			
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited									
13. SUPPLEMENTARY NOTES									
14. ABSTRACT Prostate cancer (PCa) remained the largest diagnosed and second leading cause of cancer related deaths in men in the USA. Last year alone, may have recorded deaths of 28,170 American men by castration resistant prostate cancer (CRPC) (Siegel <i>et al.</i> 2012, 2013). Therefore developing new therapeutics that improves treatment options for this disease is urgently needed.									
15. SUBJECT TERMS- none provided									
16. SECURITY CLASSIFICATION OF:						17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC	
a. REPORT U		b. ABSTRACT U		c. THIS PAGE U				19b. TELEPHONE NUMBER (<i>include area code</i>)	
						UU			

Table of Contents

	<u>Page</u>
Introduction.....	1
Body.....	3
Results.....	7
Key Research Accomplishments.....	10
Reportable Outcomes.....	10
Conclusion.....	10
Appendices.....	11
References.....	11

INTRODUCTION: W81XWH-11-1-0204

Prostate cancer (PCa) remained the largest diagnosed and second leading cause of cancer related deaths in men in the USA. Last year alone, may have recorded deaths of 28,170 American men by castration resistant prostate cancer (CRPC) (Siegel *et al.* 2012, 2013). Therefore developing new therapeutics that improves treatment options for this disease is urgently needed.

A large number of genetic, epigenetic and environmental factors contribute to the risk of prostate cancer. Among them are androgens, dietary factors, life-style related factors and genetic predisposition (Yegnasubramanian *et al.* 2004; Kim *et al.* 2012). A plethora of studies have contributed to our understanding with respect to molecular mechanisms, signaling pathways and intrinsic factors which contribute to the development of prostate cancer (PCa) and its subsequent transition to CRPC. A number of genes have been identified and characterized which are associated with inherited susceptibility of prostate cancer (De Marzo *et al.* 2003; Nelson *et al.* 2003; Nelson *et al.* 2009).

Male Androgen hormones (Testosterone, T; and Dihydrotestosterone, DHT) plays a pivotal role in the development, regulation, and maintenance of male phenotype as well as reproductive physiology and has been implicated in the development and progression of prostate cancer.

Androgen stimulates the expression of its cognate the Androgen nuclear receptor (Grossmann *et al.* 2001; Debes & Tindall 2002, 2004; Dehm & Tindall 2005, 2007). The Androgen receptor (AR) is a member of the family of intracellular steroid hormone receptors that function as transcription factor in a ligand-dependent (T and DHT) manner (Huang & Tindall 2002; Heemers *et al.* 2009; Lamont & Tindall 2010). Binding of DHT to AR facilitate the AR dimerization and the localization of the dimer protein to the nucleus. In the nucleus, AR binds to a large number of genes which contains Androgen Responsive Elements in the promoter region. The AR binding to a subset of AR-target gens triggers a transcriptional program that induces a myriad of cellular effects including proliferation, cell survival, differentiation, and secretion (Grossmann *et al.* 2001; Huang & Tindall 2002; Heemers & Tindall 2007). Emerging data suggests that constitutive sustained expression of AR may lead to activation of compensative cellular processes which facilitates the development of CRPC (Craft & Sawyers 1998; Chen *et al.* 2004; Chen *et al.* 2008). AR is prominent target for the treatment of non-organ confined prostate cancer by hormonal blockade therapy which uses anti-

androgens to competitively inhibits the binding of androgen to the ligand binding domain of the receptor (Craft & Sawyers 1998; Chen *et al.* 2008).

The AR is heterogeneously expressed in primary tumors and throughout the progression of hormone-sensitive and CRPC. In prostate carcinogenesis, changes in AR signaling pathways activate the growth of malignant cells (Chen *et al.* 2004). The hormone-refractory stage of the disease is commonly associated with the constitutive activation of AR expression by yet unknown molecular mechanisms (Craft & Sawyers 1998; Craft *et al.* 1999). In addition, it has also been suggested that AR may be targeting and modifying cellular factors to develop resistance and circumvent AR targeting therapies. A recent research suggests that AR can be targeted by andro-miRs in CRPC adjuvant therapeutics (Sikand *et al.* 2011a; Sikand *et al.* 2011b) . It has also envisioned that resistance to treatment lead to alteration of AR role which modify the intrinsic microenvironment to modify behavior of certain cellular factors (Chen *et al.* 2004; Chen *et al.* 2008; Perets *et al.* 2012; Balbas *et al.* 2013).

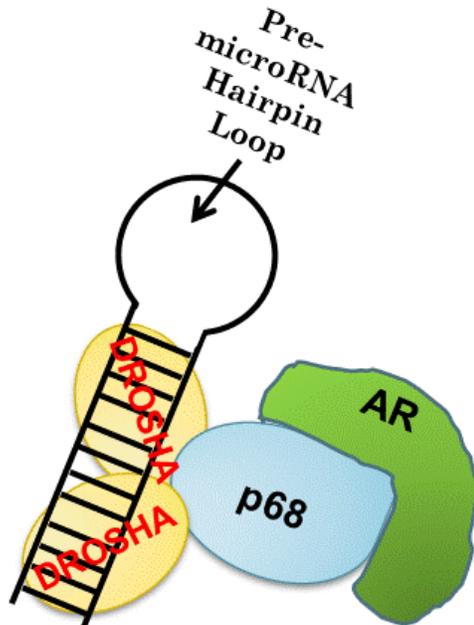
AR appears to interact with hundreds of cellular factors which include a large number of genomic loci and scores of protein factors implicated in Prostate carcinogenesis. The role of androgen and the AR appears to modulate the switching to autocrine pathway in favor of the paracrine loop. This pivotal switch appears to be played by AR interactions with cellular factors (Rajan *et al.* 2011; Perets *et al.* 2012; Vainio *et al.* 2012; Beltran *et al.* 2013; Imberg-Kazdan *et al.* 2013). One of the molecular mechanisms by which a prostate cancer cell survives and metastasizes after androgen-ablation therapy, perhaps involves the noncanonical functions of the AR including RNA processing and RNA-Protein interactions.

BODY: Based on some earlier observations that the AR was found in a complex associated with p68 helicase protein, which in turn interacts with the Drosha (Denli *et al.* 2004; Clark *et al.* 2008a; Clark *et al.* 2008b). Drosha, is a class III ribonuclease which function to process precursor-microRNAs (pre-miRNAs) to mature miRNA (Denli *et al.* 2004; Sohn *et al.* 2007; Mueller *et al.* 2010). miRNAs are class of small RNA which negatively regulates their target mRNAs by binding to the 3' UTR via hydrogen bonding. An earlier report showed that RNA helicase p68 is a coregulator of AR function in prostate cancer cells (Clark *et al.* 2008a; Clark *et al.* 2008b; Clark *et al.* 2013). Since, it is known that p68 RNA helicase interacts with the Drosha enzymes, we had proposed that AR might be modifying the post-transcriptional processing of “andro-miR” miRNAs

which have potential to contribute to the development of resistance to PCa therapies (Sikand *et al.* 2011a; Sikand *et al.* 2011b; Ebron 2013). miRNAs fine-tune gene expression at translational level by binding to its target mRNA 3' UTR and repressing protein synthesis. Cancer related miRNA studies are mainly focused on identifying miRNAs which target expression of genes implicated in various cancers including prostate cancer (PCa).

Proposal/Hypothesis: We had proposed potential protein:protein interactions among AR/p68 RNA Helicase/Drosha RNase III proteins in order to prevent the processing of “Andro-miRs,” the

SCHEMATIC OF THE PROPOSED HYPOTHESIS



The figure recapitulates the hypothesis i.e. pre-miRNA are bound to Drosha which also interacts with p68RNA helicase. P68 provides a bridge between AR and Drosha to facilitate processing of a subset of miRNAs

miRNA which are targeting AR expression.

One and only proposed specific aim and subaims of the study was **to identify miRNAs associated with AR/p68/Drosha complex.**

Stepwise, the proposed study was designed

1. to detect and confirm the existence of tripartite protein complex containing AR/p68 RNA Helicase/Drosha RNase III;
2. to optimize experimental conditions for the enrichment and isolation of the complex containing all three proteins;
3. to assay if the complex contains miRNAs;
4. and if the complex contains RNA component, we needed to establish the identify these interacting miRNAs either by miRNA microarray or by small RNA cloning and sequencing methods.

Aim: To identify miRNAs associated with AR/p68/Drosha complex: Following are sub-aims.

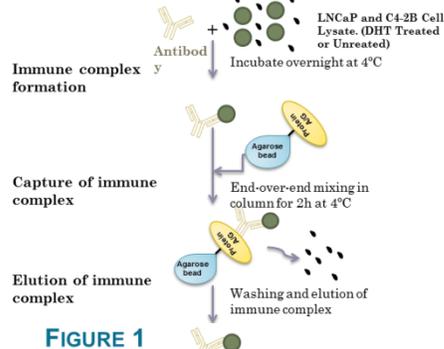
- 1a. Optimization of RIP-ChIP assay to pull-down AR/p68/Drosha complex to recover bound small RNAs.
- 1b. to clone small RNAs to generate cDNA libraries for sequencing.
- 1c. Sequencing and identification of miRNAs and miRNA microarrays to identify novel and existing known (albeit processed by AR/p68/Drosha complex) miRNAs, respectively, and
- 1d. to validate the intrinsic association properties of these miRNA with the AR/p68/Drosha complex by RNAi.

TASK 1: RNA Immunoprecipitation (RIP) of AR/p68/Drosha complex bound to primary or precursor miRNAs

- A. Optimization of RIP in LNCaP cells (Months 1-3).
- B. Optimization of RIP in C4-2B cells (Months 1-3).
- C. Isolation of AR/p68/Drosha complex enriched with pri- and/or pre-miRNAs and other noncoding RNAs (Months 3-6).

Task 1A: Optimization of RIP assay to pull-down AR/p68/Drosha complex to recover bound small RNAs.

CO-IMMUNOPRECIPITATION OF P68/ANDROGEN RECEPTOR/DROSHA COMPLEX



Western blot analysis of immunoprecipitated proteins using an antibody directed against the protein of interest

What we have done to accomplish the goals of

the Task 1a: This task was the most crucial and the other subaims were basically dependent on the successful completion of the task1a. In this task we attempted to optimize RIP/ChIP in LNCaP and C4-2B cells for several months.

During the earlier phase of the study a postdoctoral fellow attached to this project left the institute to pursue a faculty position around the starting time of the grant in 2011. Later, I

hired a graduate student in the January of 2012 consequently our projected time was delayed by several months. The student was able to optimize the Co-IP component of the RNA immunoprecipitation experiments. Here we present representative dataset from all the experiments performed to accomplish the task 1a.

Optimization and Experimental conditions for CoIP:

The CoIP experiments were done essentially following the previously published conditions (Berggard *et al.* 2007; Falsone *et al.* 2008; Jedamzik & Eckmann 2009). In brief, The cells were lysed using IP lysis buffer (Pierce Classic IP Kit - 26146). The immune complex was formed by incubating 10 µg of antibody (AR(441) sc-7305 mouse monoclonal IgG1/p68 RNA helicase(D-7) sc-365164 mouse monoclonal IgG2a , Santa Cruz Biotechnology/ Drosha (D28B1) rabbit mAb, Cell Signalling) with 500 µg of total protein lysate at 4 °C overnight. The immune complex formed with desired protein and antibody was captured using protein A/G agarose resin. The resin was washed and incubated with antibody/lysate complex in the spin column. The mixture was incubated at 4 °C with end-over-end shaking for 2h. The resin was washed to remove nonspecific proteins and the immune complex was eluted using Non reducing sample buffer. The protein complex is boiled at 100 °C and then applied on SDS-PAGE gel for further analysis.

Based on published evidences we had predicted the presumptive existence of AR/p68/Drosha complex and its putative role in processing of small RNAs (Clark *et al.* 2008a; Clark *et al.* 2008b). To test this hypothesis, first we needed to optimize and perform Co-IP using whole cell lysates from LNCaP and C4-2B cells. We pulled down protein:protein interacting complexes using one of three antibodies (AR or p68 RNA Helicase or Drosha) and probed the presence of other two proteins in Western blots. This was necessary to establish the biochemical existence of the tripartite complex is in PCa cells. We used LNCaP and C4-2B cell lines because they represent the preclinical models of AR-positive androgen-dependent and AR-positive androgen-independent stage of prostate cancer (Thalmann *et al.* 1994; Thalmann *et al.* 2000; Denmeade *et al.* 2003a; Denmeade *et al.* 2003b; Liu *et al.* 2004).

Results: Results for Co-IP using AR or p68 Helicase or Drosha are essentially the same results that we submitted last year. These results show our efforts directed towards the optimization of the protocols. The main idea of these experiments was to pull down complex containing AR/p68 RNA Helicase/Drosha with AR antibodies. To test our hypothesis we performed numerous Co-IPs using antibodies against the three proteins. If the case of the presence of tripartite proteins' complex we expected to see the detection of all three proteins using a single type antibody directed towards the one member of the complex. So in theory single antibody has potential to pulldown the tripartite

protein complex containing all three proteins.

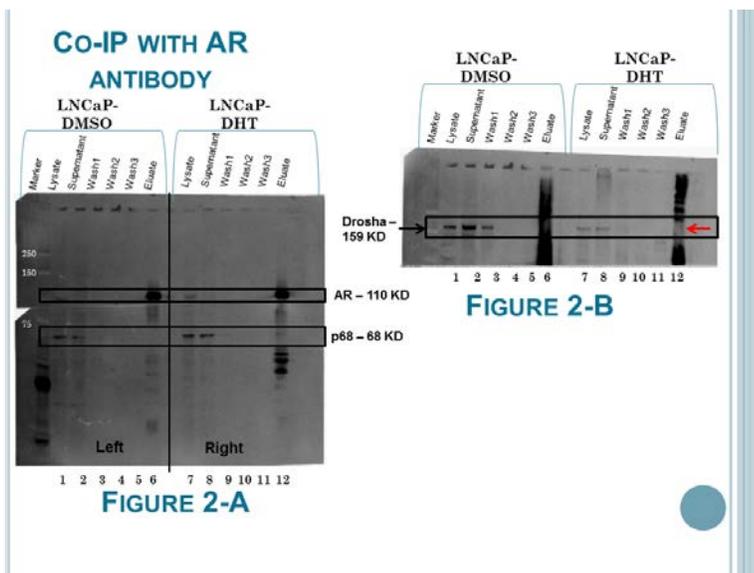


Figure 2: Results of Co-IP performed by using anti- AR antibody: Western blots were probed with anti-p68 helicase or anti-Drosha antibodies. Figure 2A-Left and right panel show the results of Co-IP done using lysates prepared from LNCaP cells treated with vehicle (DMSO) or DHT, respectively. DHT treatment was done to enrich the putative tripartite protein complex. In Right panel of Fig 2-A, Lane 12 show that p68 RNA Helicase was precipitated with AR antibody. In addition, we also observed very faint band of Drosha (expected molecular weight 159 kd) (Figure 2-B lane 12 marked

by Red Arrow).

Detection of p68 RNA helicase and Drosha protein in AR antibody pull down experiment:

First we optimized protein complex pull-downs using AR antibodies and probing the western blot using either AR (as a positive control) Drosha or p68 RNA helicase antibodies in LNCaP cell line. Cells were treated with appropriate concentrations of DHT to ensure the activation and nuclear localization of AR and enrichment of AR/p68/Drosha nuclear complexes. As results show in Figure 2, AR Co-IP pull down experiment was able to detect p68 (Fig 2, Lower Right Panel A, Lane 12 in DHT enriched LNCaP cells). In addition, we found a faint band when the blot was probed with Drosha antibody (Figure 2B, Lane 12, Red arrow). It is interesting to observe that DHT treatment was able to enrich the complex for Drosha. However, the enrichment wasn't sufficient enough to detect higher concentration of Drosha. Since the experimental conditions were mild the complex contains many other proteins that are cross reacting with Drosha antibody.

Detection of Drosha and AR protein in p68 RNA helicase antibody pull down experiment:

Next we optimized protein complex pull-downs from LNCaP cell lysates using p68 antibodies and probed the complex for the presence of AR and Drosha proteins. LNCaP and C4-2B Cells were appropriately treated with DHT for the activation of AR and enrichment of putative AR/p68/Drosha nuclear complexes. Results of the experiments are shown in the Figure 3. Figure legend describes

the details of the results.

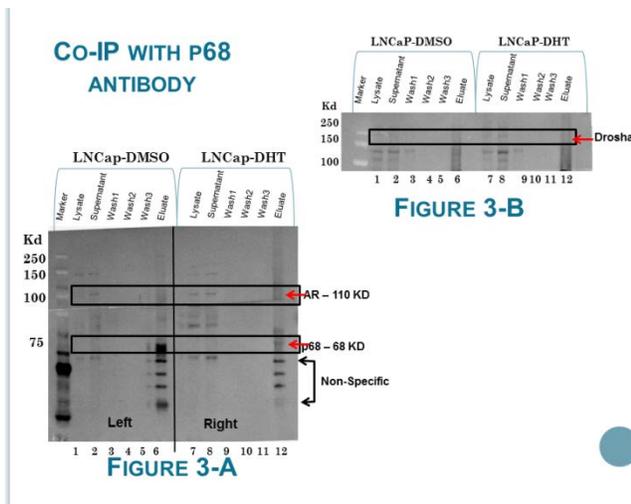


Figure 3: Detection of AR and Drosha proteins in p68 pull-down experiment: The figure shows results of Co-IP performed using anti-p68 RNA Helicase antibody. The blots were probed with anti-AR (Figure 3A-Right top LNCaP-DHT Panel, Lane 12) or with anti-Drosha (Figure 3B-LNCaP-DHT Panel, Lane 12, marked by a red arrow).

The data indicates the presence of AR and Drosha proteins in the complex as shown in Figure 3. Figure 3A-Right top LNCaP-DHT Panel, Lane 12. In addition, we also detected a

very signal for Drosha in Figure 3B-LNCaP-DHT Panel, Lane 12.

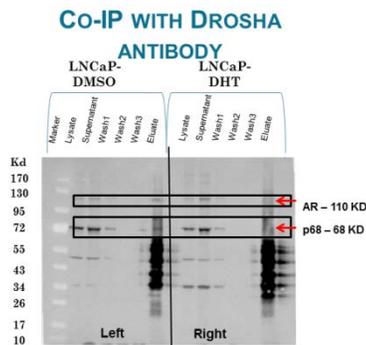


FIGURE 4

Finally, we attempted to pull-down putative AR/p68 RNA Helicase/Drosha complex using Drosha antibody and probed the blots for the presence of with AR and p68 RNA helicase.

Figure 4: The figure show results of Co-IP performed using anti-Drosha antibody. The western blots were probed with anti-AR and anti-p68 antibodies. Please see the presence of p68 band in lower red-box in LNCaP-DHT panel Elute Lane.

Summary of the Results discussed above: The above experiments suggested the presence of AR/p68 RNA helicase/Drosha RNase III enzymes in the tripartite protein:protein complex. The results show that proteins (all three) are detectable most in DHT enriched lysates. This result suggests that the protein complex is highly enriched in DHT mediated translocation of AR in nucleus. We are able to detect the presence of all three proteins in three co-IP experiments. However, the presence of Drosha in AR or presence of AR in Drosha pulldown experiments needs to be confirmed by an alternative experiment. This experiment can be performed using ectopically expressing recombinant proteins in any cell line.

TASK 2: Identification of RIP miRNAs and other noncoding RNAs: The success of this task was mainly dependent on the success of the task 1. We needed to establish conditions for highly enrichment of the tripartite protein complex. However, our repeated attempts to enrich RNA:Protein complexes from the lysates prepared from the cells were futile. Due to the lack of any Drosha protein in the proposed tri-protein complex our attempts to continue to purify RNA did not work as anticipated.

Subtasks proposed but not accomplished:

- A. Sequencing of library and identification of RNAs by bioinformatics;
- B. primary miRNA microarray; and

- C. Validation of intrinsic association of miRNA with the novel AR/p68/Drosha complex will be established by RNAi of AR and/or p68 genes.

RIP-ChIP -

RIP-ChIP was essentially performed using the conditions that are described in original protocols (Keene *et al.* 2006; Mukherjee *et al.* 2011; Erhard *et al.* 2013). To perform RIP in LNCaP or C4-2B cells to isolate small RNA, we developed a protocol to pull down and clone RNA bound to AR/p68/Drosha complex for sequencing. We cloned fraction of RNAs isolated from protein:RNA complex in TOPO vectors. However, sequencing of a large number of clones did not reveal any novel or known RNAs that we can pursue further.

Key Research Accomplishments: During the span of this grant we tested our hypothesis if AR binds to p68 and Drosha to modify processing of certain miRNAs. In this project we were able to identify interaction between the AR and p68 RNA helicase. However, we were unable to find any indication that Drosha bound to p68 also have AR bound to this complex. We made serious attempts to optimize the CO-IP protocols, however our repeated efforts did not yield any data that show triple protein complex bound to RNA. As such there are not tangible key research accomplishments that can be reported at this time.

Reportable Outcomes: We have data that indicates the intrinsic association of AR with p68 RNA helicase but not with Drosha. However, there is not enough data that could be used for any extended studies for the moment.

Conclusion: During the previous reporting session, we were able to optimize CO-IP methods to pull-down AR/p68/Drosha protein:protein interacting complex in LNCaP cells. For the past one year, we extended the study on C4-2B cells however unable to identify any novel interacting complex that we had proposed in the original grant application. We did try to isolate and clone, if any small RNAs from pull down complexes, but without any luck. We hereby conclude, that the

current data does not support our hypothesis that AR has potential to modify expression of a subset of miRNAs involved in prostate carcinogenesis.

APPENDICES: No Appendix is currently needed for this report.

REFERENCES:

- Balbas M.D., Evans M.J., Hosfield D.J., Wongvipat J., Arora V.K., Watson P.A., Chen Y., Greene G.L., Shen Y. & Sawyers C.L. (2013) Overcoming mutation-based resistance to antiandrogens with rational drug design. *Elife* 2, e00499.
- Beltran H., Yelensky R., Frampton G.M., Park K., Downing S.R., Macdonald T.Y., Jarosz M., Lipson D., Tagawa S.T., Nanus D.M., Stephens P.J., Mosquera J.M., Cronin M.T. & Rubin M.A. (2013) Targeted Next-generation Sequencing of Advanced Prostate Cancer Identifies Potential Therapeutic Targets and Disease Heterogeneity. *Eur Urol* 63, 920-6.
- Berggard T., Linse S. & James P. (2007) Methods for the detection and analysis of protein-protein interactions. *Proteomics* 7, 2833-42.
- Chen C.D., Welsbie D.S., Tran C., Baek S.H., Chen R., Vessella R., Rosenfeld M.G. & Sawyers C.L. (2004) Molecular determinants of resistance to antiandrogen therapy. *Nat Med* 10, 33-9.
- Chen Y., Sawyers C.L. & Scher H.I. (2008) Targeting the androgen receptor pathway in prostate cancer. *Curr Opin Pharmacol* 8, 440-8.
- Clark E.L., Coulson A., Dalgliesh C., Rajan P., Nicol S.M., Fleming S., Heer R., Gaughan L., Leung H.Y., Elliott D.J., Fuller-Pace F.V. & Robson C.N. (2008a) The RNA helicase p68 is a novel androgen receptor coactivator involved in splicing and is overexpressed in prostate cancer. *Cancer Res* 68, 7938-46.
- Clark E.L., Fuller-Pace F.V., Elliott D.J. & Robson C.N. (2008b) Coupling transcription to RNA processing via the p68 DEAD box RNA helicase androgen receptor co-activator in prostate cancer. *Biochem Soc Trans* 36, 546-7.
- Clark E.L., Hadjimichael C., Temperley R., Barnard A., Fuller-Pace F.V. & Robson C.N. (2013) p68/Ddx5 supports beta-catenin & RNAP II during androgen receptor mediated transcription in prostate cancer. *PLoS One* 8, e54150.
- Craft N. & Sawyers C.L. (1998) Mechanistic concepts in androgen-dependence of prostate cancer. *Cancer Metastasis Rev* 17, 421-7.
- Craft N., Shostak Y., Carey M. & Sawyers C.L. (1999) A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. *Nat Med* 5, 280-5.
- De Marzo A.M., Meeker A.K., Zha S., Luo J., Nakayama M., Platz E.A., Isaacs W.B. & Nelson W.G. (2003) Human prostate cancer precursors and pathobiology. *Urology* 62, 55-62.

- Debes J.D. & Tindall D.J. (2002) The role of androgens and the androgen receptor in prostate cancer. *Cancer Lett* 187, 1-7.
- Debes J.D. & Tindall D.J. (2004) Mechanisms of androgen-refractory prostate cancer. *N Engl J Med* 351, 1488-90.
- Dehm S.M. & Tindall D.J. (2005) Regulation of androgen receptor signaling in prostate cancer. *Expert Rev Anticancer Ther* 5, 63-74.
- Dehm S.M. & Tindall D.J. (2007) Androgen receptor structural and functional elements: role and regulation in prostate cancer. *Mol Endocrinol* 21, 2855-63.
- Denli A.M., Tops B.B., Plasterk R.H., Ketting R.F. & Hannon G.J. (2004) Processing of primary microRNAs by the Microprocessor complex. *Nature* 432, 231-5.
- Denmeade S.R., Litvinov I., Sokoll L.J., Lilja H. & Isaacs J.T. (2003a) Prostate-specific antigen (PSA) protein does not affect growth of prostate cancer cells in vitro or prostate cancer xenografts in vivo. *Prostate* 56, 45-53.
- Denmeade S.R., Sokoll L.J., Dalrymple S., Rosen D.M., Gady A.M., Bruzek D., Ricklis R.M. & Isaacs J.T. (2003b) Dissociation between androgen responsiveness for malignant growth vs. expression of prostate specific differentiation markers PSA, hK2, and PSMA in human prostate cancer models. *Prostate* 54, 249-57.
- Ebron J.S.W., C.M.; Shukla, G.C. (2013) Targeting of Androgen Receptor Expression by Andro-miRs as Novel Adjunctive Therapeutics in Prostate Cancer. *Journal of Cancer Therapy* Vol.4, 47-58.
- Erhard F., Dolken L. & Zimmer R. (2013) RIP-chip enrichment analysis. *Bioinformatics* 29, 77-83.
- Falsone S.F., Gesslbauer B. & Kungl A.J. (2008) Coimmunoprecipitation and proteomic analyses. *Methods Mol Biol* 439, 291-308.
- Grossmann M.E., Huang H. & Tindall D.J. (2001) Androgen receptor signaling in androgen-refractory prostate cancer. *J Natl Cancer Inst* 93, 1687-97.
- Heemers H.V., Regan K.M., Schmidt L.J., Anderson S.K., Ballman K.V. & Tindall D.J. (2009) Androgen modulation of coregulator expression in prostate cancer cells. *Mol Endocrinol* 23, 572-83.
- Heemers H.V. & Tindall D.J. (2007) Androgen receptor (AR) coregulators: a diversity of functions converging on and regulating the AR transcriptional complex. *Endocr Rev* 28, 778-808.
- Huang H. & Tindall D.J. (2002) The role of the androgen receptor in prostate cancer. *Crit Rev Eukaryot Gene Expr* 12, 193-207.
- Imberg-Kazdan K., Ha S., Greenfield A., Poultney C.S., Bonneau R., Logan S.K. & Garabedian M.J. (2013) A genome-wide RNA interference screen identifies new regulators of androgen receptor function in prostate cancer cells. *Genome Res* 23, 581-91.
- Jedamzik B. & Eckmann C.R. (2009) Analysis of RNA-protein complexes by RNA coimmunoprecipitation and RT-PCR analysis from *Caenorhabditis elegans*. *Cold Spring Harb Protoc* 2009, pdb prot5300.
- Keene J.D., Komisarow J.M. & Friedersdorf M.B. (2006) RIP-Chip: the isolation and identification of mRNAs, microRNAs and protein components of ribonucleoprotein complexes from cell extracts. *Nat Protoc* 1, 302-7.
- Kim J.W., Kim S.T., Turner A.R., Young T., Smith S., Liu W., Lindberg J., Egevad L., Gronberg H., Isaacs W.B. & Xu J. (2012) Identification of new differentially

- methylated genes that have potential functional consequences in prostate cancer. *PLoS One* 7, e48455.
- Lamont K.R. & Tindall D.J. (2010) Androgen regulation of gene expression. *Adv Cancer Res* 107, 137-62.
- Liu A.Y., Brubaker K.D., Goo Y.A., Quinn J.E., Kral S., Sorensen C.M., Vessella R.L., Beldegrun A.S. & Hood L.E. (2004) Lineage relationship between LNCaP and LNCaP-derived prostate cancer cell lines. *Prostate* 60, 98-108.
- Mueller G.A., Miller M.T., Derosé E.F., Ghosh M., London R.E. & Hall T.M. (2010) Solution structure of the Drosha double-stranded RNA-binding domain. *Silence* 1, 2.
- Mukherjee N., Corcoran D.L., Nusbaum J.D., Reid D.W., Georgiev S., Hafner M., Ascano M., Jr., Tuschl T., Ohler U. & Keene J.D. (2011) Integrative regulatory mapping indicates that the RNA-binding protein HuR couples pre-mRNA processing and mRNA stability. *Mol Cell* 43, 327-39.
- Nelson W.G., De Marzo A.M. & Isaacs W.B. (2003) Prostate cancer. *N Engl J Med* 349, 366-81.
- Nelson W.G., De Marzo A.M. & Yegnasubramanian S. (2009) Epigenetic alterations in human prostate cancers. *Endocrinology* 150, 3991-4002.
- Perets R., Kaplan T., Stein I., Hidas G., Tayeb S., Avraham E., Ben-Neriah Y., Simon I. & Pikarsky E. (2012) Genome-wide analysis of androgen receptor targets reveals COUP-TF1 as a novel player in human prostate cancer. *PLoS One* 7, e46467.
- Rajan P., Dalglish C., Carling P.J., Buist T., Zhang C., Grellscheid S.N., Armstrong K., Stockley J., Simillion C., Gaughan L., Kalna G., Zhang M.Q., Robson C.N., Leung H.Y. & Elliott D.J. (2011) Identification of novel androgen-regulated pathways and mRNA isoforms through genome-wide exon-specific profiling of the LNCaP transcriptome. *PLoS One* 6, e29088.
- Siegel R., Naishadham D. & Jemal A. (2012) Cancer statistics, 2012. *CA Cancer J Clin* 62, 10-29.
- Siegel R., Naishadham D. & Jemal A. (2013) Cancer statistics, 2013. *CA Cancer J Clin* 63, 11-30.
- Sikand K., Barik S. & Shukla G.C. (2011a) MicroRNAs and Androgen Receptor 3' Untranslated Region: A Missing Link in Castration-resistant Prostate Cancer? *Mol Cell Pharmacol* 3, 107-13.
- Sikand K., Slaibi J.E., Singh R., Slane S.D. & Shukla G.C. (2011b) miR 488* inhibits androgen receptor expression in prostate carcinoma cells. *Int J Cancer* 129, 810-9.
- Sohn S.Y., Bae W.J., Kim J.J., Yeom K.H., Kim V.N. & Cho Y. (2007) Crystal structure of human DGCR8 core. *Nat Struct Mol Biol* 14, 847-53.
- Thalmann G.N., Anezinis P.E., Chang S.M., Zhou H.E., Kim E.E., Hopwood V.L., Pathak S., von Eschenbach A.C. & Chung L.W. (1994) Androgen-independent cancer progression and bone metastasis in the LNCaP model of human prostate cancer. *Cancer Res* 54, 2577-81.
- Thalmann G.N., Sikes R.A., Wu T.T., Degeorges A., Chang S.M., Ozen M., Pathak S. & Chung L.W. (2000) LNCaP progression model of human prostate cancer: androgen-independence and osseous metastasis. *Prostate* 44, 91-103 Jul 1;44(2).
- Vainio P., Wolf M., Edgren H., He T., Kohonen P., Mpindi J.P., Smit F., Verhaegh G., Schalken J., Perala M., Iljin K. & Kallioniemi O. (2012) Integrative genomic,

transcriptomic, and RNAi analysis indicates a potential oncogenic role for FAM110B in castration-resistant prostate cancer. *Prostate* 72, 789-802.

Yegnasubramanian S., Kowalski J., Gonzalgo M.L., Zahurak M., Piantadosi S., Walsh P.C., Bova G.S., De Marzo A.M., Isaacs W.B. & Nelson W.G. (2004) Hypermethylation of CpG islands in primary and metastatic human prostate cancer. *Cancer Res* 64, 1975-86.