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TITLE: Identification of Novel Drug Targets and Lead Compounds for Advanced Prostate Cancer through Genomic and Cheminformatic Analyses

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<b>14. ABSTRACT</b> Prostate cancer (PCa) is one of the most common cancers for males in the United States and Canada. When PCa is diagnosed early, it can be treated by surgery or radiation. However, in about 30% of cases when PCa recurs or metastasizes, the disease is managed primarily by hormone therapy. Unfortunately, the effectiveness of this therapy is temporary due to emerging resistance mechanisms. Thus, the limited effectiveness and side effects of current PCa treatments demand for new therapeutics to treat advanced PCa. With the accumulated publicly available genomic data and recent advancements in bioinformatic and cheminformatic algorithms, this research project aims to apply comprehensive analyses of cancer genomes and transcriptomes to identify new drug targets, followed by the discovery of lead compounds through virtual screening techniques and biological assays. The outcome of this project can lead to new generations of anti-PCa drugs to treat cases where the cancer cells have developed resistance to existing drugs, by targeting other alternative pathways.					
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## **1. INTRODUCTION**

Prostate cancer (PCa) is one of the most common cancers for males in the United States and Canada, with 245,000 estimated cases and 32,000 deaths in 2015<sup>1,2</sup>. When PCa is diagnosed early, it can often be treated by surgery or radiation. However, in about 30% of cases when PCa recurs and/or metastasizes, the disease is managed primarily by the hormone therapy that reduces the level of androgens or blocks their binding to the androgen receptor (AR). Unfortunately, the effectiveness of this therapy is temporary due to emerging resistance mechanisms related to AR. While several chemo- and immunotherapies are available for managing such advanced PCa, they offer only modest survival benefits. Because of the limited effectiveness and significant side effects of current PCa treatments, there is a pressing demand for new therapeutics to be developed to treat aggressive and resistant prostate cancer. With the accumulated publicly available genomic data and recent advancements in bioinformatic and cheminformatic algorithms, this research project aims to apply comprehensive analyses of cancer genomes and transcriptomes to identify new drug targets, followed by the discovery of lead compounds through virtual screening techniques and biological assays. The outcome of this project can lead to new generations of anti-PCa drugs to treat cases where the cancer cells have developed resistance to existing drugs, by targeting other alternative pathways.

## **2. KEYWORDS**

Prostate cancer

Metastatic castration-resistant prostate cancer

Cancer-specific aberrations

Genomics

Transcriptomics

Proteomics

Bioinformatics

Cheminformatics

Computer-aided drug design

Small molecule inhibitors

*In silico* compound screening

*In vitro* biological assays

Compound validation

### 3. ACCOMPLISHMENTS

#### What were the major goals of the project?

#### **Year 1 (August 1, 2014 to July 31, 2015), reporting period**

<b>Specific Aim 1 - Genomics: identification of prostate cancer-related aberrations in gene regulation.</b>	Proposed dates (month/year)	Actual completion dates (month/year)
<b>Major Goal 1:</b> Collect available genomic data from prostate tumor and normal cells.	Aug/2014 - Nov/2014	Aug/2014 - Nov/2014
<u>Milestone 1:</u> Construction of a shared and central database of genomic data for prostate cancer research at Vancouver Prostate Centre, University of British Columbia	Nov/2014	Nov/2014
<b>Major Goal 2:</b> Analyze the genomic data to identify prostate cancer-specific aberrations.	Dec/2014 - May/2015	Dec/2014 - May/2015
<b>Major Goal 3:</b> Identify new drug targets in prostate cancer.	June/2015 - July/2015	June/2015 - July/2015
<u>Milestone 2:</u> Identification of three drug targets in prostate cancer.	July/2015	July/2015
<u>Milestone 3:</u> One co-author manuscript on bioinformatic analyses of genomic data and drug target identification in prostate cancer.	July/2015	Dec/2014

#### **Year 2 (August 1, 2015 to July 31, 2016)**

<b>Specific Aim 2 - Cheminformatics: development of new classes of anti-prostate cancer drugs.</b>	Proposed dates (month/year)	Actual completion dates (month/year)
<b>Major Goal 4:</b> Construct protein structural models and identify potential drug binding sites for each of three targets.	Aug/2015 - Nov/2015	
<u>Milestone 4:</u> Determine binding pockets of each of the three targets for subsequent virtual screening.	Nov/2015	
<b>Major Goal 5:</b> Virtual compound screening against the binding pocket of drug target #1.	Dec/2015 - Feb/2016	
<u>Milestone 5:</u> Identify 200 compounds with high binding potential against drug target #1 for further biological validation.	Feb/2016	
<b>Major Goal 6:</b> Virtual compound screening against the binding pocket of drug target #2.	Feb/2016 - Apr/2016	

<u>Milestone 6</u> : Identify 200 compounds with high binding potential against drug target #2 for further biological validation.	Apr/2016	
<b>Major Goal 7</b> : Virtual compound screening against the binding pocket of drug target #3.	Apr/2016 - June/2016	
<u>Milestone 7</u> : Identify 200 compounds with high binding potential against drug target #3 for further biological validation.	June/2016	
<b>Major Goal 8</b> : Compound validations by biological assays.	Aug/2015 - July/2016	
<u>Milestone 8</u> : Identify lead compounds with high biological activity against drug target #1, #2 and #3.	June/2016 - July/2016	
<u>Milestone 9</u> : Up to three co-author manuscripts on lead compounds against drug target #1, #2 and #3.	July/2016	

### **What was accomplished under these goals?**

As outlined in the previous section and in the approved SOW, the three major goals for the Specific Aim 1, “Genomics: identification of prostate cancer-related aberrations in gene regulation”, have been accomplished during the reporting period, Year 1 (August 1, 2014 to July 31, 2015). The three accomplished goals are: 1) collect available genomic data from prostate tumor and normal cells; 2) analyze the genomic data to identify prostate cancer-specific aberrations; and 3) identify new drug targets in prostate cancer.

### **Major Goal 1: Collect available genomic data from prostate tumor and normal cells.**

#### *1) Major activities.*

Publicly available PCa genomic and transcriptomic datasets from high-throughput experiments have been collected from a number of sources including the Gene Expression Omnibus (GEO)<sup>3</sup> and The Cancer Genome Atlas (TCGA)<sup>4</sup>. The public available datasets were downloaded through the corresponding web portals. In collaboration with Dr. Anna Lapuk, in-house high-quality datasets previously derived from the patient cohort at the Vancouver Prostate Centre (VPC), University of British Columbia (UBC) have been collected. Both the public and VPC datasets have been stored in a centralized hard drive on the shared VPC network. All the datasets have been checked for data quality and consistency (with respect to their original publications). These data files have been converted and stored as the following three formats: 1) tab-delimited text files for easy importing to other analysis programs; 2) data tables in the MySQL database for fast data searching; and 3) data objects in the R computational environment for all the subsequent statistical analyses. A number of scripts based on the PERL programming language have been created for data processing and cross-referencing among the difference datasets.

### 2) Specific objectives

The main objective of Major Goal 1 is to create a comprehensive collection of publicly and internally available PCa genomic and transcriptomic datasets not only for the analyses performed in this project, but also for future usages by other colleagues at the VPC, UBC.

### 3) Significant results/key outcomes

As shown in **Table 1**, this comprehensive collection of datasets consists of 2 DNA-Seq, 5 RNA-Seq, 12 microarrays and 6 ChIP-Seq datasets from a total of 1435 samples, including multiple prostate cancer cell lines. In particular, this collection also contains a high-quality in-house dataset with matched DNA-Seq and RNA-Seq data from 26 prostate tumours and 5 normal prostate samples from the patients<sup>5</sup>.

**Table 1. Prostate cancer (PCa) genomic and transcriptomic datasets collected.**

Source/Accession	Data type	Total number of samples	Sample type	PubMed ID
TCGA	DNA-Seq, RNA-Seq, SNP array	550	Patient samples	
GEO/GSE38674	DNA-Seq	2	LnCaP	23245995
VPC	RNA-Seq	31	Patient samples	25155515
GEO/GSE14092	RNA-Seq	3	LNCaP	20478527
	RNA-Seq	42	Patient samples	22389870
GEO/GSE34780	RNA-Seq	6	LNCaP	23034120
GEO/GSE38676	RNA-Seq	2	LNCaP	23245995
MSKCC/GEO/GSE21034	microarray	370	Patient samples	20579941
GEO/GSE60771	microarray	16	VCaP	25263440
GEO/GSE58940	microarray	4	VCaP	
GEO/GSE53994	microarray	15	VCaP	24591637
GEO/GSE15091	microarray	7	PC3	19668381
GEO/GSE14028	microarray	15	LNCaP, VCaP	20478527
GEO/GSE28948, GSE35540	microarray	12	VCaP	22531786
GEO/GSE36549	microarray	14	LNCaP, VCaP	22710436
GEO/GSE15805	microarray	155	LNCaP	22955617
GEO/GSE28680	microarray	24	Patient samples	23260764
GEO/GSE39654	microarray	16	LNCaP-1F5, VCaP	23269278

GEO/GSE11428	microarray	21	LnCaP	
GEO/GSE14092	ChIP-Seq	57	LNCaP, VCaP, patient samples	20478527
GEO/GSE20042	ChIP-Seq	5	LNCaP	20208536
GEO/GSE28950	ChIP-Seq	15	VCaP	22531786
GEO/GSE38682, GSE38683, GSE38684	ChIP-Seq	6	LNCaP	23245995
GEO/GSE28219	ChIP-Seq	25	LNCaP, VCaP, patient samples	23260764
GEO/GSE39879	ChIP-Seq	22	LNCaP-1F5, VCaP	23269278

## **Major Goal 2: Analyze the genomic data to identify prostate cancer-specific aberrations.**

### *1) Major activities.*

In order to identify PCa-specific aberrations, the datasets collected from Major Goal 1 have been analyzed by using the following computational algorithms. Single nucleotide variants derived from the DNA-Seq data have been annotated by the gSearch program<sup>6</sup>, and copy number variations were identified by the use of BIC-seq<sup>7</sup>. Gene fusion events have been identified by using DeFuse<sup>8</sup> on the RNA-Seq data. Differentially expressed genes from the microarray and RNA-seq data were determined by using t-test for two group comparison (tumor vs. normal). In particular, overexpressed genes were identified from each dataset by the following steps: 1) log<sub>2</sub> transformation; 2) two sample t-test between tumour and normal samples; 3) multiple testing correction on p-values<sup>9</sup>; 4) selection of genes with corrected (adjusted) p-values < 0.05; and 5) among those with significant p-values, selection of genes with fold-change  $\geq 2$  (tumor vs. normal). Transcription factor binding data from ChIP-Seq have been analyzed by using the CompleteMOTIFs pipeline<sup>10</sup>. Additional analyses on the TCGA dataset were performed by using the computational tools available at cBioPortal<sup>11</sup>.

### *2) Specific objectives*

The main objective of Major Goal 2 is to perform comprehensive analyses on the different datasets (DNA-Seq, RNA-Seq, microarray, ChIP-Seq) collected previously from Major Goal 1. The results from these analyzes have been used to identify a list of candidate drug targets for prostate cancer (more details in Major Goal 3).

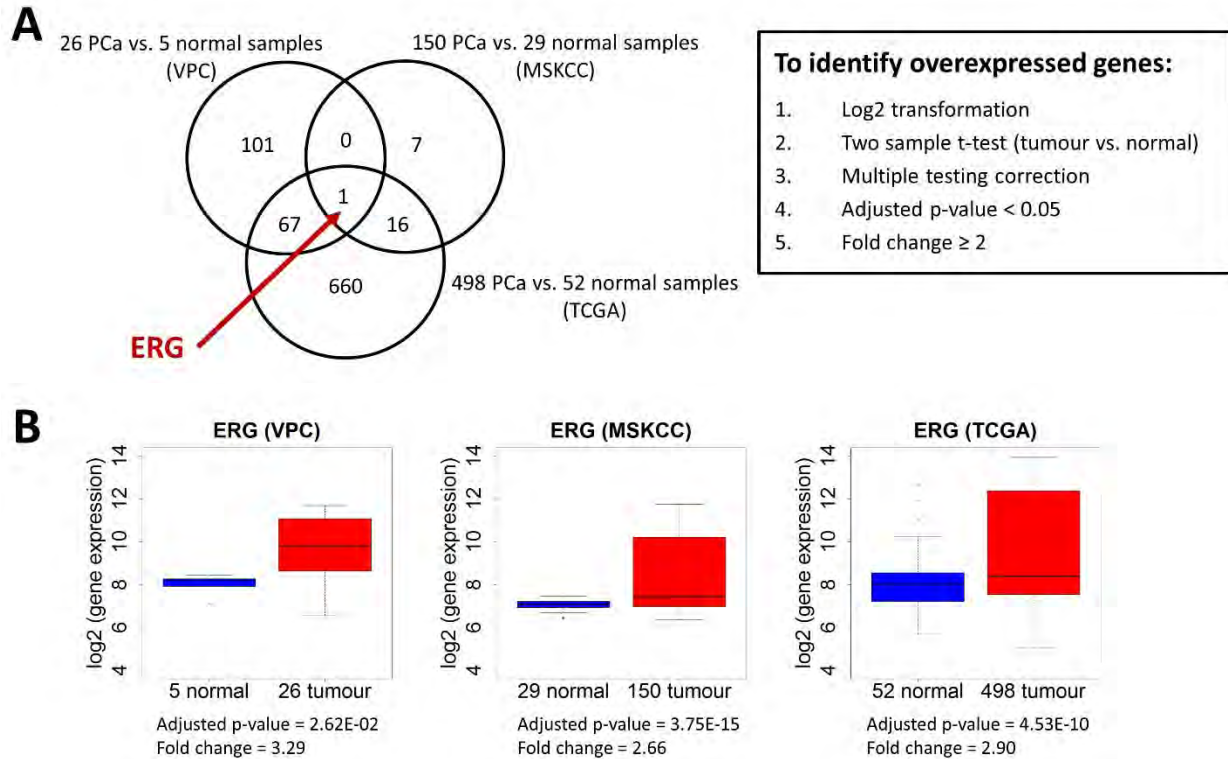
### *3) Significant results/key outcomes*

The bioinformatic analyses performed on the TCGA prostate cancer dataset, consisted of 498 PCa patient tumour samples and 52 normal samples, have identified a list of frequent genomic alterations, as shown in **Table 2**. Among the top alterations are: gene fusions involving ERG, ETV1 and ETV4; PTEN deletion; amplifications of genes including MYC, AR, and PIK3CA. The frequencies of these genomic alternations identified from the analyses is comparable to those reported in previous studies<sup>12,13</sup>.

**Table 2. Frequent genomic alterations in PCa patient samples (TCGA dataset).**

Gene	Type of alterations	% of cases in the TCGA dataset
ERG	Gene fusion	46%
PTEN	Deletion	17%
NKX3-1	Deletion	17%
TMPRSS2	Deletion	16%
ETV1	Gene fusion	10%
TP53	Deletion	8%
MYC	Amplification	8%
AR	Amplification	7%
FOXA1	Amplification	6%
ETV4	Gene fusion	5%
PIK3CA	Amplification	5%

In addition to the genomic alterations listed above, gene expression analyses performed on the different datasets collected have revealed a number of genes that are overexpressed in PCa patient samples. **Figure 1** illustrates the results from gene expression analyses performed on the two largest datasets, MSKCC<sup>14</sup> and TCGA<sup>4</sup>, as well as the in-house dataset from VPC<sup>5</sup>. Using a standard but stringent bioinformatic protocol, a total of 169, 24, 744 genes have been found to overexpress in the VPC, MSKCC and TCGA datasets, respectively (**Figure 1A**). While each dataset features a different list of genes, ERG is the only common gene overexpressed in all of the three datasets.

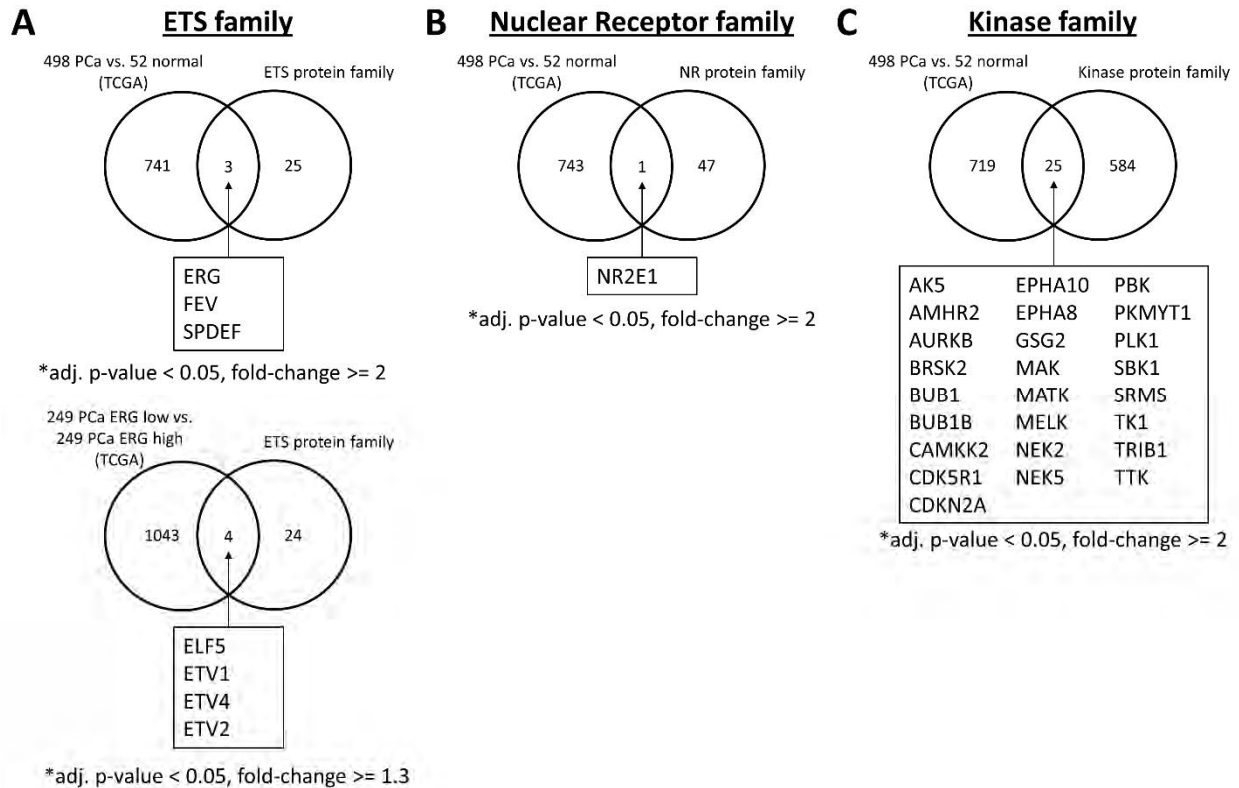


**Figure 1. ERG is one of the most commonly overexpressed genes in PCa patient samples.**

A) A Venn diagram that shows the number of overexpressed (i.e. upregulated) genes from each of the three gene expression datasets: Vancouver Prostate Centre (VPC)<sup>5</sup>, Memorial Sloan-Kettering Cancer Center (MSKCC)<sup>14</sup>, and The Cancer Genome Atlas (TCGA)<sup>4</sup>, based on a bioinformatic protocol as listed on the right panel. ERG is the only overexpressed gene from intersecting the three datasets.

B) The fold changes of ERG gene expression in PCa tumour samples, compared to normal samples, range from 2.66 to 3.29.

To further explore other overexpressed genes (in addition to ERG), the above stringent bioinformatic protocol that required a gene to be overexpressed in all three datasets has been relaxed. **Figure 2** highlights other overexpressed genes in the TCGA dataset, which represents the largest PCa gene expression data currently available, in the three major gene families of interest: 1) ETS factors, 2) nuclear receptors, and 3) kinases. In addition, we have compared the gene expression profiles between samples with low ERG expression and those with high ERG expression within the total PCa dataset from TCGA (**Figure 2A**, bottom panel). Notably, ETV1 and ETV4 are overexpressed in PCa samples where ERG is expressed at lower level. This gene overexpression is likely due to the gene fusion involving ETV1 and ETV4, and it suggests that the different ETS factors (ERG, ETV1 and ETV4) may compensate the function of each other.



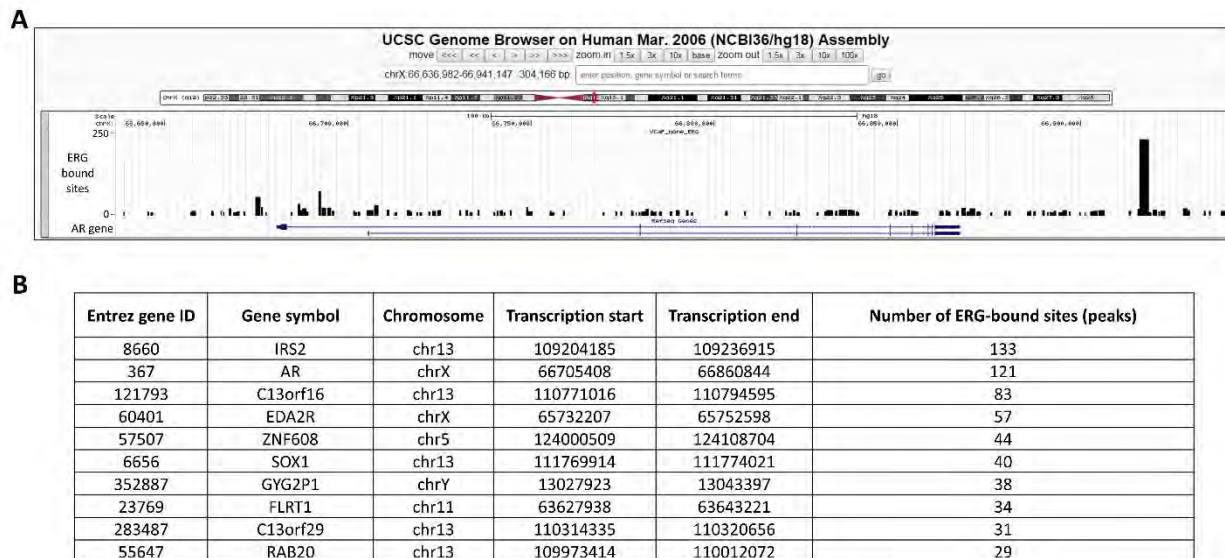
**Figure 2. Overexpressed genes from the ETS, Nuclear Receptor (NR) and Kinase families in PCa patient samples.**

A) Top panel: A list of ETS genes (ERG, FEV, SPDEF) are overexpressed in the 498 PCa samples, compared to the 52 normal samples in the TCGA dataset. Bottom panel: Within the 498 PCa samples, 4 ETS genes are overexpressed in samples that have lower ERG expression.

B) NR2E1 gene from the nuclear receptor family is overexpressed in the 498 PCa samples.

C) A number of kinase genes are overexpressed in the 498 PCa samples.

To gain more insights on DNA binding sites by the ERG transcription factor on chromosomes and nearby genes, several publicly available ChIP-Seq datasets have been analyzed. **Figure 3** features the result from analyzing one of the largest ChIP-Seq datasets<sup>15</sup>, consisted of a total of 42,568 DNA sites bound by the ERG transcription factor in VCaP cells (a PCa cell line that harbours the ERG gene fusion and overexpresses the ERG protein). All of the 42,568 ERG-bound sites have been analyzed using the CompleteMOTIFs program<sup>10</sup>, and **Figure 3B** shows the top nearby genes ranked by the number of ERG-bound sites. Interestingly, the gene Androgen Receptor (AR) is ranked as the second. Further visualization of the ERG-bound sites at the AR locus, using the UCSC Genome Browser<sup>16</sup>, illustrated that the sites, with various amount of binding intensity (ranged from 0-250), span across the entire AR gene including both upstream and downstream regions (**Figure 3A**). This result suggests that overproduction of the ERG protein may have a significant influence on the AR transcription and the AR-regulated pathways in prostate cancer cells.



**Figure 3. Enrichment of ERG-bound sites at the Androgen Receptor (AR) gene locus based on ChIP-Seq analyses.**

A) Visualization of the intensity of ERG-bound sites (y-axis) at the AR gene locus (x-axis) on the UCSC Genome Browser.

B) The top 10 genes, ranked by the number of nearby ERG-bound sites.

### Major Goal 3: Identify new drug targets in prostate cancer.

#### 1) Major activities.

By analyzing the genomic and transcriptomic datasets as summarized in Major Goal 2, we have identified a number of target genes with aberrations such as genomic alterations and gene overexpression in patient PCa samples. In order to perform an initial assessment of structural availability and druggability on these potential targets, the protein sequence of each target was queried against the Protein Data Bank (PDB)<sup>17</sup> using Protein Blast<sup>18</sup>. Protein targets with resolved x-ray crystal or NMR structures were identified, and in other cases, those with homologous structural templates ( $\geq 70\%$  sequence identity) suitable for subsequent homology modeling were determined. The program, Modeller v9.11 (with the ‘allmodel’ protocol)<sup>19</sup>, was used to build 3D protein models for the potential targets, based on the structural templates identified from PDB. Then each protein model was subjected to the Site Finder algorithm, implemented in the Molecular Operating Environment (MOE) 2012, which used virtual atomic probes to search the protein surface for suitable small molecule binding pockets<sup>20</sup>. Each pocket was scored based on its size/depth, solvent exposure, and the surrounding amino acid residues.

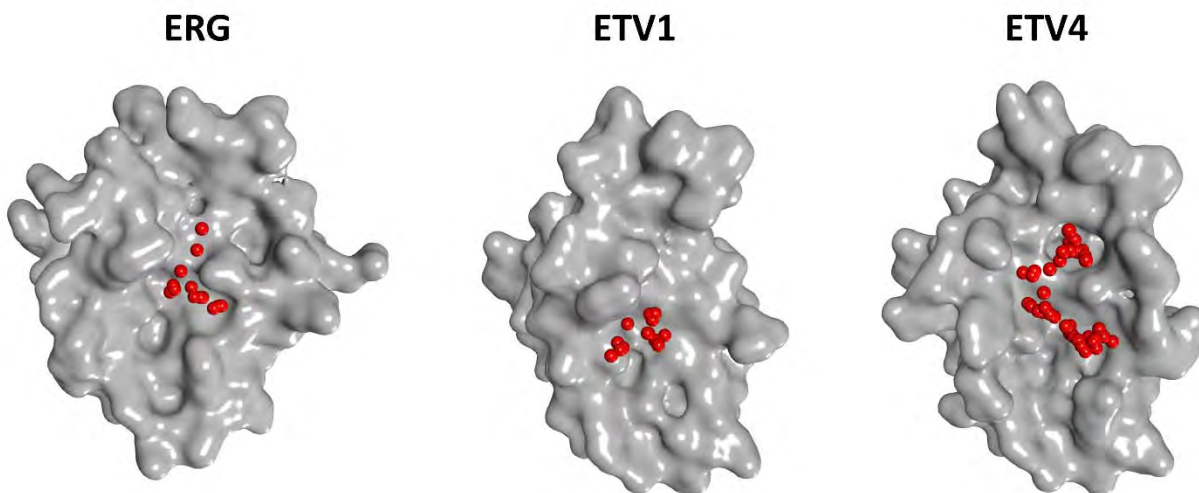
In addition to the two criteria as described above, PCa-specific aberrations and structural availability/druggability, a comprehensive literature has been performed on each potential target. In particular, we examined experimental results from previous studies that support 1) gain of cancerous phenotypes due to the overexpression of the target and/or 2) loss of cancerous features due to the knockdown of the target. All these information were presented to Dr. Paul Rennie (Mentor of this project), and together we have identified a total of three candidate drug targets that are suitable for Specific Aim 2 in Year 2, “Cheminformatics: development of new classes of anti-prostate cancer drugs”.

## 2) Specific objectives

The main objective of Major Goal 3 is to narrow the list of potential drug targets down to three candidates, based on multiple lines of supporting evidence based on 1) PCa-specific aberrations as identified from Major Goal 2; 2) existing literature supporting the oncogenic functions of the drug targets; 3) expert knowledge from Dr. Rennie; and 4) structural availability and druggability. Preliminary protein modeling and initial binding site assessment have been performed on each of the drug target candidates. More comprehensive and in-depth structural evaluations based on advanced methods such as molecular dynamics will be performed in Year 2 (Major Goal 4: Construct protein structural models and identify potential drug binding sites for each of three targets).

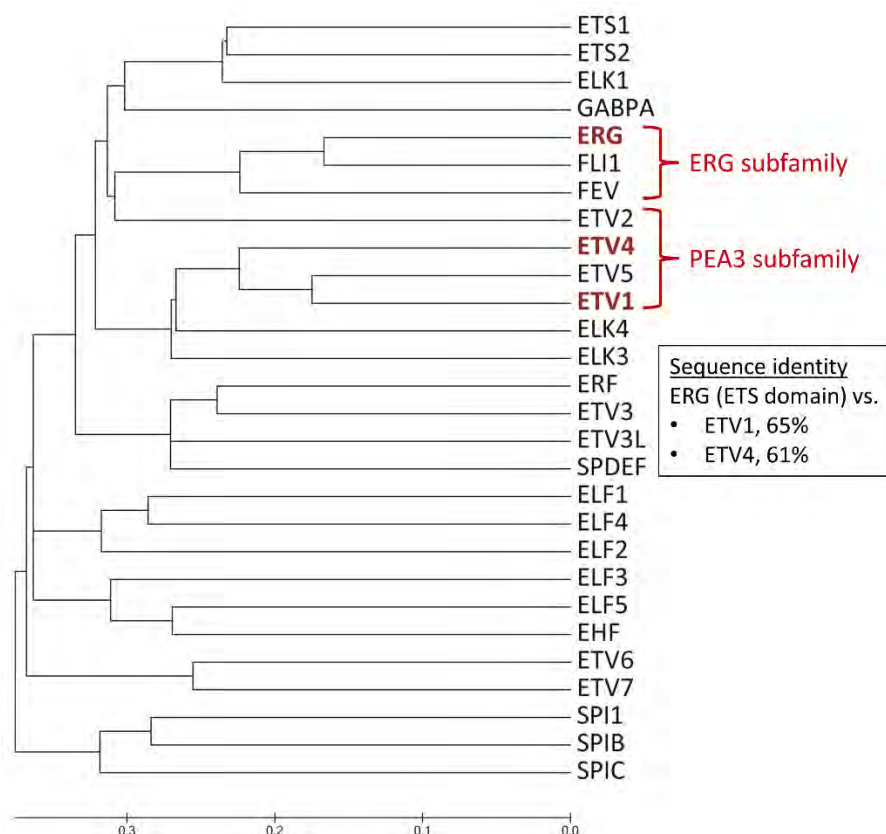
## 3) Significant results/key outcomes

Initial assessments on protein structures and druggability have been performed on a number of genes with PCa-specific aberrations, with a particular emphasis on the three gene families: ETS factors, nuclear receptors and kinases. **Figure 4** shows suitable drug-binding sites identified on the surfaces of ERG, ETV1 and ETV4 protein models. To further examine the similarity and difference among the three ETS protein sequences, a phylogenetic tree has been constructed using a multiple sequence alignment program, Clustal Omega<sup>21</sup>. As shown in **Figure 5**, the ETS family consists of a total of 28 members, and ERG and ETV1/4 are clustered to the ERG and PEA3 subfamilies, respectively. In addition, the sequence alignment indicates that ETV1/4 shares 61-65% of sequence identity with ERG; thus, it is feasible to develop specific small molecules targeting each of the three ETS factors by exploiting their sequence and structural differences.



**Figure 4. Initial assessment of druggable sites on the three drug target candidates.**

Surfaces of predicted protein models for the three drug target candidates: ERG, ETV1 and ETV4. Potential drug-binding sites were predicted by using virtual atomic probes (shown as red spheres).



**Figure 5. ETS phylogenetic tree.**

A phylogenetic tree of the 28 members in the ETS family, based on a full-length multiple protein sequence alignment.

Combining all the analysis results from the three major goals (genomic alteration, gene overexpression, protein structure availability and druggability, as well as supporting evidence accumulated in the literature<sup>12,13,22-25</sup> (**Table 3**), the three ETS factors (ERG, ETV1 and ETV4) have been identified as the top drug target candidates to be moved forward into Year 2 of the project, for the “Specific Aim 2 - Cheminformatics: development of new classes of anti-prostate cancer drugs”.

**Table 3. A summary of the three drug target candidates identified.**

Drug target	Genomic alteration	Gene overexpression	Protein structure availability	Druggability	Supporting evidence in literature
ERG	Gene fusion	Fold-change $\geq 2$	yes	yes	yes
ETV1	Gene fusion	Fold-change $\geq 1.3$	yes	yes	yes
ETV4	Gene fusion	Fold-change $\geq 1.3$	yes	yes	yes

#### *4) Other achievements*

The bioinformatic analyses on the nuclear receptors, in particular the orphan nuclear receptors, as drug targets in prostate cancer have resulted in a review paper<sup>26</sup>. A manuscript that focuses on the ETS factors as drug targets in prostate cancer is currently in preparation.

### **What opportunities for training and professional development has the project provided?**

#### *Training activities:*

This project has provided me (Dr. Michael Hsing, postdoctoral fellow) a great amount of opportunities for training and professional development. In order to enhance my knowledge in the field of cancer research (and in particular, prostate cancer), I have received personalized training from my mentor, Dr. Paul Rennie, who has over 30 years of research experience in prostate cancer and more than 180 peer-reviewed publications principally in this field. The training involved literature review of previous and current research on various topics in prostate cancer and other cancers as guided by Dr. Rennie. Cancer-related journal articles and textbooks were recommended by Dr. Rennie for studying. My knowledge in the field of prostate cancer and other cancers in general have been examined during weekly in-person meetings and email communication with Dr. Rennie. Furthermore, I have applied this knowledge on prostate cancer to the writing of a review article<sup>26</sup> in this field, and I have assisted my mentor, Dr. Paul Rennie, for preparing and submitting two research grant applications. One has been successfully awarded by the Pacific Northwest Prostate Cancer SPORE program, while the other has been submitted to the Canadian Cancer Society Research Institute. In addition, I have gained experience on analyzing high-throughput sequencing data by working with Dr. Anna Lapuk and other bioinformatic scientists at VPC, UBC.

#### *Professional development activities:*

Vancouver Prostate Centre (VPC), UBC is located at the largest research hub in Vancouver, adjacent to the Vancouver General Hospital and its affiliated research centers, BC Cancer Agency (BCCA), and the Genome Science Centre. This research environment greatly facilitates research communication and collaboration among not only local scientists but also international researchers through regular seminars. I have learned about the latest cancer research findings and interacted with international cancer research scientists through a number of seminar series including weekly Terry Fox seminars hosted at VPC, bi-weekly seminars at BCCA, and monthly SPORE video conferences hosted by the Fred Hutchinson Cancer Research Center at Seattle, Washington. I have presented my research findings at regular group meetings at VPC, and I attended bi-weekly journal clubs at VPC. In addition, I have attended and presented a poster at an international conference, American Association for Cancer Research (AACR) Annual Meeting at Philadelphia during April 18-22, 2014.

### **How were the results disseminated to communities of interest?**

Nothing to Report.

### **What do you plan to do during the next reporting period to accomplish the goals?**

In accordance with the approved SOW, I have accomplished all the major goals and tasks in this reporting period (Year 1: Specific Aim 1 - Genomics: identification of prostate cancer-related aberrations in gene regulation.). A total of three drug target candidates have been identified, and the project is on track for the next reporting period (Year 2: Specific Aim 2 - Cheminformatics:

development of new classes of anti-prostate cancer drugs). In order to accomplish the goals for Year 2, I will follow the plan as described in the approved SOW. In particular, the following goals will be achieved: 1) construction of protein structural models and identification of potential drug binding sites for each of three targets; 2) virtual compound screening against the binding pockets for each of the three drug targets; and 3) compound validations by biological assays (in collaboration with Dr. LeBlanc).

## 4. IMPACT

### **What was the impact on the development of the principal discipline(s) of the project?**

Prostate cancer (PCa) is one of the most common cancers for males in the United States and Canada, with 245,000 estimated cases and 32,000 deaths in 2015<sup>1,2</sup>. Major issues facing PCa patients are cancer recurrence due to the presence of distant metastasis at time of surgery and the development of treatment resistance. The proposed project is aimed to address this urgent issue of metastasis and drug-resistance in prostate cancer by using the combined power of genomic analyses, protein structural modeling, computer-aided drug design and biological screening to develop new classes of anti-prostate cancer drugs, targeting previously under-appreciated proteins that play important roles in cancer progression.

To address a Prostate Cancer Research Program (PCRP) overarching challenge, “Develop effective treatments and address mechanisms of resistance for men with high risk or metastatic prostate cancer” with a focus area, “Therapy: Identification of new targets, pathways, and therapeutic modalities”, the project has identified three drug targets based on large-scale and comprehensive analyses of available genomic and transcriptomic data in prostate tumor samples (as a result of the current reporting period, Year 1). During the next reporting period (Year 2), each of the drug targets will be screened rigorously against millions of virtual chemical models to identify candidate molecules with the best structural fit in the binding sites. The activity of these selected compounds will then be validated by various biological assays in collaborations with research scientists at Vancouver Prostate Centre (VPC), University of British Columbia.

Although it is a lengthy and costly process to take a drug from a laboratory to a patient (with an average time of 10-15 years and a cost up to billions of dollars), the time and cost will be significantly reduced by the computational approaches proposed by the project and the unique integration at VPC, combining drug discovery and preclinical development under a single research center. We anticipate that results from this project will lead to new drugs that can be used alternatively or synergistically with current therapies to benefit patients with the most deadly and advanced forms of prostate cancer.

### **What was the impact on other disciplines?**

Nothing to Report.

### **What was the impact on technology transfer?**

Nothing to Report.

### **What was the impact on society beyond science and technology?**

Nothing to Report.

## 5. CHANGES/PROBLEMS

### **Changes in approach and reasons for change**

Nothing to Report.

### **Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to Report.

### **Changes that had a significant impact on expenditures**

Nothing to Report.

### **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to Report.

### **Significant changes in use or care of human subjects**

Nothing to Report.

### **Significant changes in use or care of vertebrate animals.**

Nothing to Report.

### **Significant changes in use of biohazards and/or select agents**

Nothing to Report.

## 6. PRODUCTS

### **Publications, conference papers, and presentations**

#### **Journal publications.**

Roshan-Moniri M, Hsing M, Butler MS, Cherkasov A, Rennie PS. Orphan nuclear receptors as drug targets for the treatment of prostate and breast cancers. *Cancer Treat Rev.* (2014). 40: 1137-52. (Published).

#### **Books or other non-periodical, one-time publications.**

Nothing to Report.

#### **Other publications, conference papers, and presentations.**

##### *Oral presentations:*

Hsing M. Development of small molecule inhibitors of ERG to treat advanced prostate cancer. Prostate Centre/ Terry Fox Seminar Series. Jim Pattison Pavilion, Vancouver General Hospital, Vancouver, Canada. February 13, 2015. (Local). Acknowledgement of federal support (yes).

##### *Poster presentations and abstracts:*

Roshan-Moniri M, Hsing M, Butler MS, Lau D, Axerio-Cilies P, Yen P, Kim A, Lien S, Mroczek M, Ma D, Li H, Guo Y, Ban F, Ghaidi F, LeBlanc E, McIntosh L, Cox ME, Cherkasov A, and Rennie P. Therapeutic targeting of ETS factor ERG for the treatment of prostate cancer. AACR Annual Meeting 2015. Pennsylvania Convention Center, Philadelphia, USA. April 18-22, 2015. (International). Acknowledgement of federal support (yes).

Butler MS, Hsing M, Roshan-Moniri M, Lau D, Yen P, Kim A, Lien S, Mroczek M, Ghaidi F, LeBlanc E, McIntosh L, Cox ME, Cherkasov A, and Rennie PS. Targeting ETS factor ETV4 as a novel therapeutic for the management of breast and prostate cancer. AACR Annual Meeting 2015. Pennsylvania Convention Center, Philadelphia, USA. April 18-22, 2015. (International). Acknowledgement of federal support (yes).

### **Website(s) or other Internet site(s)**

Nothing to Report.

### **Technologies or techniques**

Nothing to Report.

### **Inventions, patent applications, and/or licenses**

Nothing to Report.

### **Other Products**

A central shared database, consisted of all the genomic and transcriptomic datasets collected during this reporting period, has been constructed and is accessible by other research scientists at the Vancouver Prostate Centre, University of British Columbia.

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

### What individuals have worked on the project?

Name:	Michael Hsing
Project Role:	PI, Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	Dr. Hsing has performed work in all the areas, as described in the Year 1 of the approved SOW.
Funding Support:	

Name:	Paul Rennie
Project Role:	Mentor, Professor, Director
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Rennie has provided the mentorship and guidance to Dr. Hsing over the course of the project.
Funding Support:	Canadian Cancer Society Research Institute, Canadian Institute of Health Research, Terry Fox Foundation

Name:	Anna Lapuk
Project Role:	Collaborator, Research Scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Lapuk has provided RNA-Seq data from the Vancouver Prostate Centre, University of British Columbia.
Funding Support:	Canadian Cancer Society Research Institute

### Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

**What other organizations were involved as partners?**

Nothing to Report.

**8. SPECIAL REPORTING REQUIREMENTS**

None required (as indicated in the Award document).

**COLLABORATIVE AWARDS:**

**QUAD CHARTS:**

## 9. APPENDICES

### References

- [1]. American Cancer Society. Prostate cancer statistics. <http://www.cancer.org/cancer/prostatecancer/detailedguide/prostate-cancer-key-statistics>
- [2]. Canadian Cancer Society. Prostate cancer statistics. <http://www.cancer.ca/en/cancer-information/cancer-type/prostate/statistics/?region=sk>
- [3]. Barrett T, Troup DB, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Muetter RN, Holko M, Ayanbule O, Yefanov A, Soboleva A. NCBI GEO: archive for functional genomics data sets--10 years on. *Nucleic Acids Res.* (2011). 39: D1005-10.
- [4]. The Cancer Genome Atlas. Prostate Adenocarcinoma. <http://cancergenome.nih.gov/cancersselected/prostatecancer>
- [5]. Wyatt AW, Mo F, Wang K, McConeghy B, Brahmabhatt S, Jong L, Mitchell DM, Johnston RL, Haegert A, Li E, Liew J, Yeung J, Shrestha R, Lapuk AV, McPherson A, Shukin R, Bell RH, Anderson S, Bishop J, Hurtado-Coll A, Xiao H, Chinnaiyan AM, Mehra R, Lin D, Wang Y, Fazli L, Gleave ME, Volik SV, Collins CC. Heterogeneity in the inter-tumor transcriptome of high risk prostate cancer. *Genome Biol.* (2014). 15: 426.
- [6]. Song T, Hwang KB, Hsing M, Lee K, Bohn J, Kong SW. gSearch: a fast and flexible general search tool for whole-genome sequencing. *Bioinformatics.* (2012). 28: 2176-7.
- [7]. Xi R, Hadjipanayis AG, Luquette LJ, Kim TM, Lee E, Zhang J, Johnson MD, Muzny DM, Wheeler DA, Gibbs RA, Kucherlapati R, Park PJ. Copy number variation detection in whole-genome sequencing data using the Bayesian information criterion. *Proc Natl Acad Sci U S A.* (2011). 108: E1128-36.
- [8]. McPherson A, Hormozdiari F, Zayed A, Giuliany R, Ha G, Sun MG, Griffith M, Heravi Moussavi A, Senz J, Melnyk N, Pacheco M, Marra MA, Hirst M, Nielsen TO, Sahinalp SC, Huntsman D, Shah SP. deFuse: an algorithm for gene fusion discovery in tumor RNA-Seq data. *PLoS Comput Biol.* (2011). 7: e1001138.
- [9]. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B-Methodological.* (1995). 57: 289-300.
- [10]. Kuttippurathu L, Hsing M, Liu Y, Schmidt B, Maskell DL, Lee K, He A, Pu WT, Kong SW. CompleteMOTIFs: DNA motif discovery platform for transcription factor binding experiments. *Bioinformatics.* (2011). 27: 715-7.
- [11]. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C, Schultz N. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal.* (2013). 6: p11.
- [12]. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, Lee C, Montie JE, Shah RB, Pienta KJ, Rubin MA, Chinnaiyan AM. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science.* (2005). 310: 644-8.
- [13]. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, Montgomery B, Taplin ME, Pritchard CC, Attard G, Beltran H, Abida W, Bradley RK, Vinson J, Cao X, Vats P, Kunju LP, Hussain M, Feng FY, Tomlins SA, Cooney KA, Smith DC, Brennan C, Siddiqui J, Mehra R, Chen Y, Rathkopf DE, Morris MJ, Solomon SB, Durack JC, Reuter VE, Gopalan A,

- Gao J, Loda M, Lis RT, Bowden M, Balk SP, Gaviola G, Sougnez C, Gupta M, Yu EY, Mostaghel EA, Cheng HH, Mulcahy H, True LD, Plymate SR, Dvinge H, Ferraldeschi R, Flohr P, Miranda S, Zafeiriou Z, Tunariu N, Mateo J, Perez-Lopez R, Demichelis F, Robinson BD, Schiffman M, Nanus DM, Tagawa ST, Sigaras A, Eng KW, Elemento O, Sboner A, Heath EI, Scher HI, Pienta KJ, Kantoff P, de Bono JS, Rubin MA, Nelson PS, Garraway LA, Sawyers CL, Chinnaiyan AM. Integrative clinical genomics of advanced prostate cancer. *Cell*. (2015). 161: 1215-28.
- [14]. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, Arora VK, Kaushik P, Cerami E, Reva B, Antipin Y, Mitsiades N, Landers T, Dolgalev I, Major JE, Wilson M, Socci ND, Lash AE, Heguy A, Eastham JA, Scher HI, Reuter VE, Scardino PT, Sander C, Sawyers CL, Gerald WL. Integrative genomic profiling of human prostate cancer. *Cancer Cell*. (2010). 18: 11-22.
- [15]. Yu J, Mani RS, Cao Q, Brenner CJ, Cao X, Wang X, Wu L, Li J, Hu M, Gong Y, Cheng H, Laxman B, Vellaichamy A, Shankar S, Li Y, Dhanasekaran SM, Morey R, Barrette T, Lonigro RJ, Tomlins SA, Varambally S, Qin ZS, Chinnaiyan AM. An integrated network of androgen receptor, polycomb, and TMPRSS2-ERG gene fusions in prostate cancer progression. *Cancer Cell*. (2010). 17: 443-54.
- [16]. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D. The human genome browser at UCSC. *Genome Res*. (2002). 12: 996-1006.
- [17]. Rose PW, Bi C, Bluhm WF, Christie CH, Dimitropoulos D, Dutta S, Green RK, Goodsell DS, Prlic A, Quesada M, Quinn GB, Ramos AG, Westbrook JD, Young J, Zardecki C, Berman HM, Bourne PE. The RCSB Protein Data Bank: new resources for research and education. *Nucleic Acids Res*. (2013). 41: D475-82.
- [18]. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol*. (1990). 215: 403-10.
- [19]. Eswar N, Webb B, Marti-Renom MA, Madhusudhan MS, Eramian D, Shen MY, Pieper U, Sali A. Comparative protein structure modeling using Modeller. *Curr Protoc Bioinformatics*. (2006). Chapter 5: Unit 5 6.
- [20]. Group CC. Chemical Computing Group: Molecular Operating Environment. <http://www.chemcomp.com/>
- [21]. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol*. (2011). 7: 539.
- [22]. Rubin MA, Maher CA, Chinnaiyan AM. Common gene rearrangements in prostate cancer. *J Clin Oncol*. (2011). 29: 3659-68.
- [23]. Aytes A, Mitrofanova A, Kinkade CW, Lefebvre C, Lei M, Phelan V, LeKaye HC, Koutcher JA, Cardiff RD, Califano A, Shen MM, Abate-Shen C. ETV4 promotes metastasis in response to activation of PI3-kinase and Ras signaling in a mouse model of advanced prostate cancer. *Proc Natl Acad Sci U S A*. (2013). 110: E3506-15.
- [24]. Rahim S, Uren A. Emergence of ETS transcription factors as diagnostic tools and therapeutic targets in prostate cancer. *Am J Transl Res*. (2013). 5: 254-68.
- [25]. Feng FY, Brenner JC, Hussain M, Chinnaiyan AM. Molecular pathways: targeting ETS gene fusions in cancer. *Clin Cancer Res*. (2014). 20: 4442-8.
- [26]. Roshan-Moniri M, Hsing M, Butler MS, Cherkasov A, Rennie PS. Orphan nuclear receptors as drug targets for the treatment of prostate and breast cancers. *Cancer Treat Rev*. (2014). 40: 1137-52.



## Anti-Tumour Treatment

## Orphan nuclear receptors as drug targets for the treatment of prostate and breast cancers

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## ABSTRACT

Nuclear receptors (NRs), a family of 48 transcriptional factors, have been studied intensively for their roles in cancer development and progression. The presence of distinctive ligand binding sites capable of interacting with small molecules has made NRs attractive targets for developing cancer therapeutics. In particular, a number of drugs have been developed over the years to target human androgen- and estrogen receptors for the treatment of prostate cancer and breast cancer. In contrast, orphan nuclear receptors (ONRs), which in many cases lack known biological functions or ligands, are still largely under investigated. This review is a summary on ONRs that have been implicated in prostate and breast cancers, specifically retinoic acid-receptor-related orphan receptors (RORs), liver X receptors (LXRs), chicken ovalbumin upstream promoter transcription factors (COUP-TFs), estrogen related receptors (ERRs), nerve growth factor 1B-like receptors, and “dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1” (DAX1). Discovery and development of small molecules that can bind at various functional sites on these ONRs will help determine their biological functions. In addition, these molecules have the potential to act as prototypes for future drug development. Ultimately, the therapeutic value of targeting the ONRs may go well beyond prostate and breast cancers.

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## Introduction

The nuclear receptor (NR) superfamily is composed of transcriptional factors that enable multicellular organisms to regulate gene expression in response to a wide variety of stimuli from developmental, physiological and environmental sources. NR actions are modulated by several endogenous mechanisms that include: (1) ligand binding, (2) posttranslational modification of amino acid residues, (3) protein dimerization, (4) nuclear transfer, (5) protein–protein interactions with activators and repressors, and (6) cooperative DNA binding with other transcription factors [1]. Of the 48 members of the superfamily, 36 receptors were originally described as orphan NRs (ONRs) because their exact function and native ligands were undefined [2]. As a result of recent research

efforts, 21 of these ONRs became annotated as “adopted” due to the discovery of natural or synthetic ligands (Fig. 1A).

NRs are commonly classified into 7 subfamilies based on their sequence similarity [3] and have been studied for their associations with cancers in a wide range of human organs (Table 1). However, there is a disparity of research efforts dedicated to ONRs, as illustrated by the number of published articles in PubMed (Fig. 1B). For example, during the year 2013 alone, 543 and 1312 articles were published on the androgen receptor (AR) and estrogen receptor (ER) in the context of their corresponding cancers. By comparison, less than 30 articles were published in the same year on the role of each ONR in cancer, as highlighted by Fig. 1C. This imbalance in research activity is also mirrored in the number of drugs and chemicals targeting the ONRs (Fig. 1A).

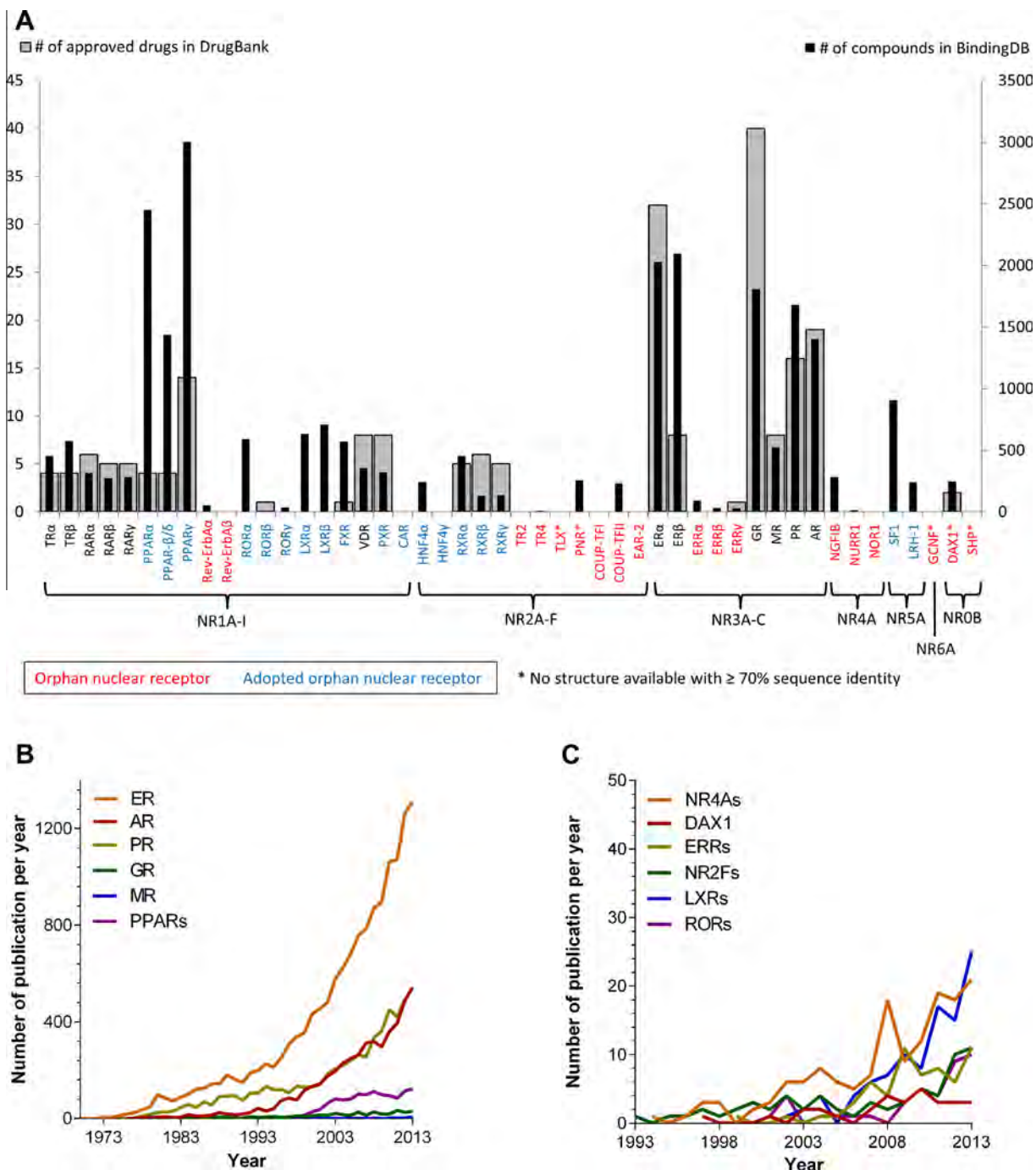
Conventionally, targeting transcriptional factors has been a challenging task in drug discovery due to the lack of well-defined active sites [4]. Thus, the unique presence of the ligand binding domain in NRs has made them very attractive drug targets, and NRs are among the top four families of drug targets and account for approximately 13% of all Food and Drug Administration (FDA)-approved drugs [5]. To gain insight into the number of existing small molecules targeting the NR family, each NR was queried based on its gene symbol, protein name and protein sequence

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<sup>1</sup> Co-first authors.

<sup>2</sup> The Rennie and Cherkasov labs contributed equally to this work.



**Fig. 1.** Comparison of existing approved drugs, experimental compounds and publications for NR and ONRs. (A) Each NR was queried for the number of approved drugs (the left y-axis shown in gray bar) in DrugBank [6], and for the number of published compounds (the right y-axis shown in black bar) with experimentally-determined binding affinities in BindingDB [7] in October 2013. The NRs are arranged according to the subfamily as appeared in Table 1, and further classified as orphan or adopted ONR according to Shi et al. [2] Their protein sequences were compared with those with existing experimental structures in PDB [25] by using standard protein BLAST [40]. NRs that have no structural template with sequence identity higher than 70% are marked by \*. The number of articles published on (B) the five hormone receptors (ER, AR, PR, GR and MR) and PPARs and (C) the ONRs discussed in this review paper, were extracted from PubMed in December 2013 using a search query consisted of: (Cancer [Title/Abstract]) AND ("Name of the nuclear receptor"[Title/Abstract]). Gene names and synonyms were used wherever applicable.

through the DrugBank [6] and BindingDB [7] databases. The DrugBank contains information on existing drugs and their direct targets, including 1541 FDA-approved small molecule therapeutics and 5082 experimental drugs that interact with 4323 proteins. BindingDB currently contains over one million binding affinity records corresponding to 427,325 small molecules and 6589 protein targets, extracted from the literature. As illustrated in Fig. 1, five hormone NRs (AR, ER, glucocorticoid receptor (GR),

mineralocorticoid receptor (MR), and progesterone receptor (PR)) are associated with a large number of synthetic inhibitors developed for the treatment of prostate- and breast cancers among other diseases [8,9]. Similarly, some adopted ONR such as peroxisome proliferator-activated receptors (PPARs) have received considerable attention and have clinically approved drugs for the treatment of diabetes and obesity [2]. In contrast, most other members of the ONR family have fewer associated inhibitors identified due to

**Table 1**  
Nuclear receptors in cancers.

Subfamily	Group name	Abbreviation	Cancer association	
1. Thyroid hormone receptor-like	Thyroid hormone receptor (NR1A)	TR $\alpha$ TR $\beta$	Liver [166], Kidney [167], Pituitary [168], Breast [169], Thyroid [170]	
	Retinoic acid receptor (NR1B)	RAR $\alpha$ RAR $\beta$ RAR $\gamma$	Leukemia [171], Breast [172], Head & Neck [173], Lung [174], Oral tissue [175], Cervix [176], Ovary [177], Esophageal [178], Prostate [179]	
	Peroxisome proliferator-activated receptor (NR1C)	PPAR $\alpha$ PPAR- $\beta/\delta$ PPAR $\gamma$	Brain [180], Lung [181], Stomach & Colon [182], Liver [183], Pancreas [184], Bladder [185], Breast [186], Testis [187], Bone [188]	
	Rev-ErbA (NR1D)	Rev-ErbA $\alpha$ Rev-ErbA $\beta$	N/A	
	RAR-related orphan receptor (NR1F)	ROR $\alpha$ ROR $\beta$ ROR $\gamma$	Lymphoma [189], Breast [73], Prostate [69]	
	Liver X receptor-like (NR1H)	LXR $\alpha$ LXR $\beta$ FXR	Prostate [87], Breast [190], Colon [191], Pancreas [192], Esophageal [193], Liver [194]	
	Vitamin D receptor-like (NR1I)	VDR PXR CAR	Colon [195], Prostate [196], Breast [197] Colon [198], Breast [199], Ovary [200] Liver [201]	
	2. Retinoid X receptor-like	Hepatocyte nuclear factor (NR2A)	HNF4 $\alpha$ HNF4 $\gamma$	Liver [202], Colon [203], Breast [204], Prostate [205]
		Retinoid X receptor (NR2B)	RXR $\alpha$ RXR $\beta$ RXR $\gamma$	Breast [146] Colon [206]
		Testicular receptor (NR2C)	TR2 TR4	N/A
TLX/PNR (NR2E)		TLX PNR	N/A	
COUP/EAR (NR2F)		COUP-TFI COUP-TFII EAR-2	Prostate [106], Lung [207] Breast [108]	
3. Estrogen receptor-like		Estrogen receptor (NR3A)	ER $\alpha$ ER $\beta$	Breast [9], Cervix [208], Colon [209], Liver [210], Lung [211], Pancreas [212], Prostate [213], Thyroid [214], Esophageal [215], Ovary [216], Adrenocortical [217]
	Estrogen related receptor (NR3B)	ERR $\alpha$ ERR $\beta$ ERR $\gamma$	Prostate [115], Breast [218]	
	3-Ketosteroid receptors (NR3C)	GR MR PR AR	Prostate [219], Breast [220], Lung [221], Pancreas [222] N/A Ovary [223], Endometrial [224], Gallbladder [225], Breast [226] Prostate [8], Breast [227]	
	4. Nerve growth factor 1B-like	NGFIB/NURR1/NOR1 (NR4A)	NGFIB NURR1 NOR1	Breast [228], Colon [229], Prostate [143]
5. Steroidogenic factor-like		SF1/LRH1 (NR5A)	SF1 LRH-1	Prostate [230], Adrenocortical Tumors [231] Breast [232], Colon [233]
	6. Germ cell nuclear factor-like	GCNF (NR6A)	GCNF	N/A
0. Miscellaneous	DAX/SHP (NR0B)	DAX1 SHP	Bone [234], Breast [155], Prostate [121]	

Nomenclature used is based on the system defined by the Nuclear Receptors Nomenclature Committee [235].

difficulties in drug development and toxicity, as well as, the continued focus on the identification of natural ligands [10]. For example, the role of retinoid X receptors (RXRs) as heterodimeric partners of several members of the nuclear receptor family, including retinoic acid receptor (RAR), makes them promising drug targets; however, controversy still surrounds whether or not 9-*cis*-retinoic acid is its true endogenous ligand [11].

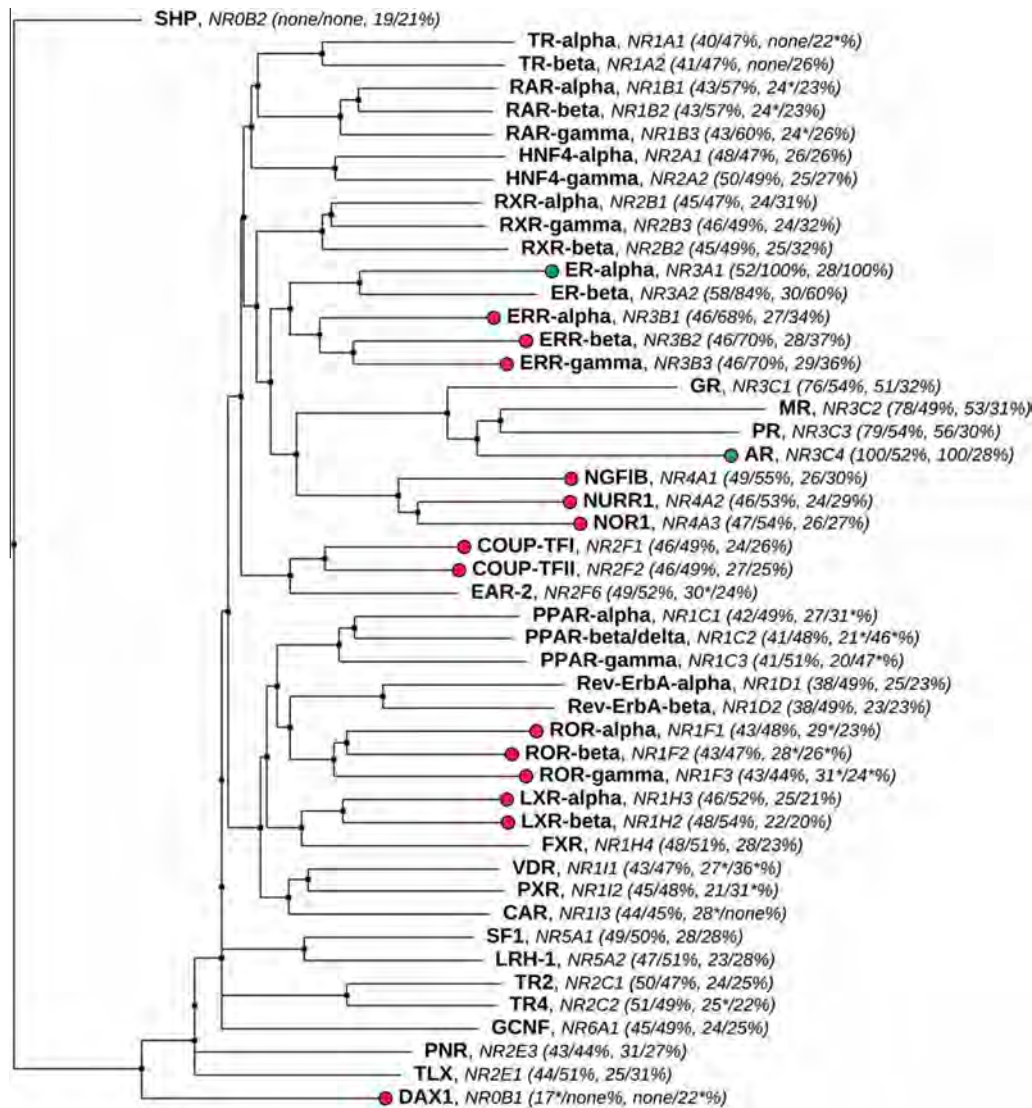
To bring attention to ONRs as prospective novel cancer drug targets, this article reviews general structural features and biological functions of ONRs and discusses some of the most promising candidates for therapeutic targeting in both prostate and breast cancers.

### Targeting different functional sites in NRs with small molecules

All NRs are modular in nature and composed of six conserved regions, designated A-F, which make up the following domains: (A/B) the N-terminal regulatory domain (NTD), (C) DNA binding

domain (DBD), (D) hinge region, (E) ligand binding domain (LBD), and (F) C-terminal domain. A phylogenetic tree was constructed based on a multiple alignment of full-length protein sequences of all 48 NRs using the Clustal Omega [12] software, and it demonstrated that each NR can be further grouped into the corresponding subfamilies based on sequence similarities (Fig. 2). In addition, Fig. 2 features sequence identity between each NR and AR/ER at the DBD and LBD, and it highlights that DBDs are more conserved, sharing ~50% sequence identity, while the LBD is less conserved (~25% sequence identity). The extent of sequence conservation within the DBD and LBD regions are further depicted as a heat map in Fig. 3A.

Among the six domains, the NTD of nuclear receptors contains a transcriptional activation function-1 site (AF-1), and its activity does not directly depend on the presence of activating ligands [13,14]. Although the transcriptional activity of AF-1 is limited, it is known to synergize with the ligand-regulated activation



**Fig. 2.** A phylogenetic tree of NRs. A phylogenetic tree was constructed based on a multiple alignment of full-length protein sequences of all 48 NRs using Clustal Omega [12]. The NRs featured in the review paper are highlighted in red, and the two nuclear receptors, AR and ER $\alpha$ , are indicated in green as the reference. The sequence identity of each NR is compared to AR and ER $\alpha$  at the DBD and LBD, with the domain boundary positions defined by NCBI Conserved Domain Database (CDD) [161]. The following format is used (a/b%, c/d%): a = % identity vs. AR-DBD, b = % identity vs. ER $\alpha$ -DBD, c = % identity vs. AR-LBD, and d = % identity vs. ER $\alpha$ -LBD. \* = query alignment coverage is less than 50%.

function-2 (AF-2) regulatory site located within the LBD to regulate gene expression. Significant variability in the NTD can be observed even within a single type of NR where different sequences can be derived from alternative splicing, promoters, and transcription start sites. One of the best examples of such variability is found with RAR where three gene templates, designated  $\alpha$ ,  $\beta$  and  $\gamma$  yield eight alternative protein variants [15,16].

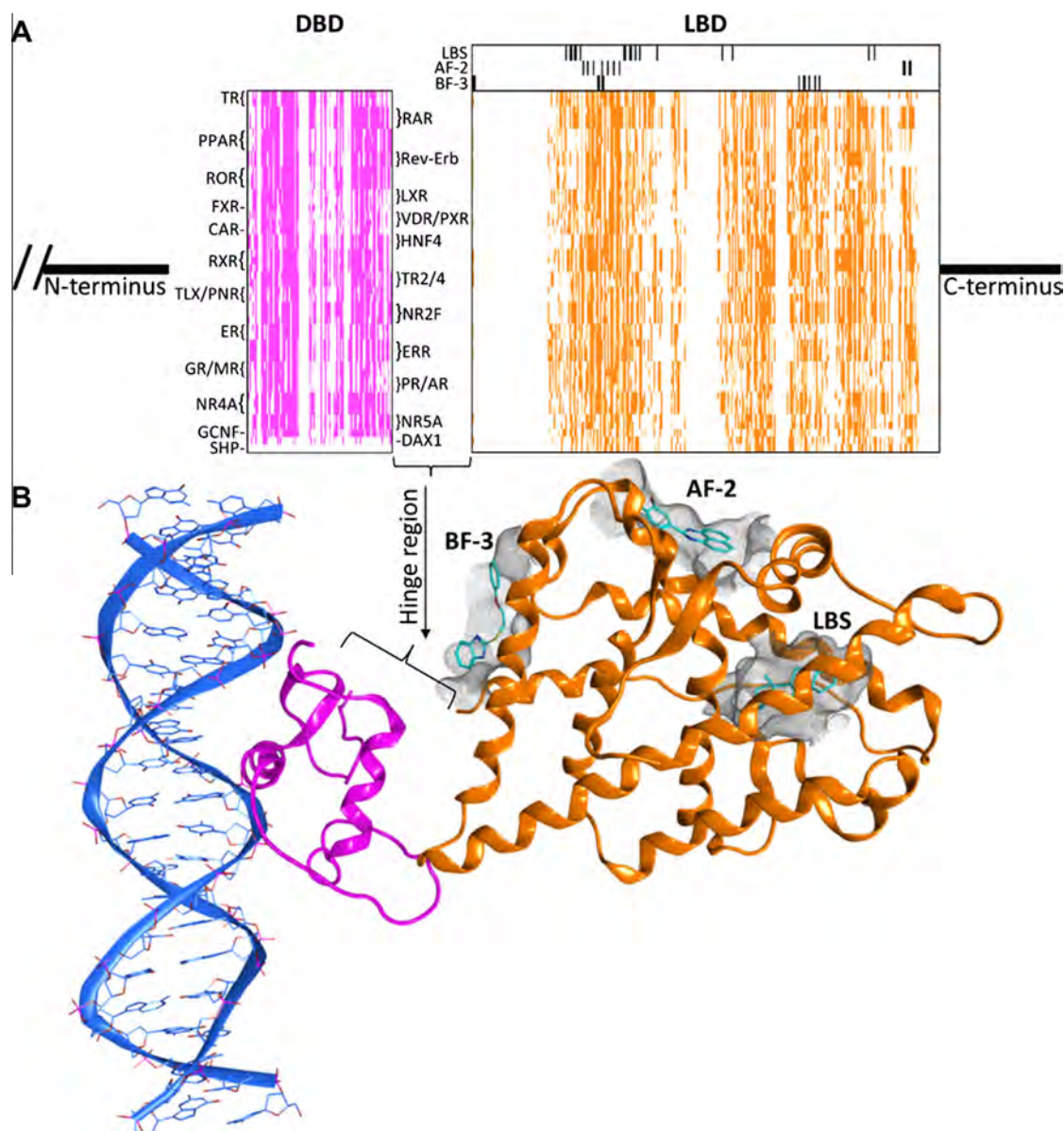
The DBD has the highest sequence conservation among the NRs, and it mediates receptor binding to response elements (REs) located in the enhancers and promoters upstream of NR-regulated genes. The receptors can bind to REs as a monomer, homodimer or heterodimer, and the two zinc finger motifs within the DBD aid RE binding and receptor dimerization [17]. All NRs contain a DBD, with the exception of dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1 (DAX1) and small heterodimer partner (SHP), both of which lack a DNA binding domain [18,19]. The absence of DBD on DAX1 and SHP can also be demonstrated by the lack of sequence alignment in Fig. 3A.

The hinge region is the connecting bridge between the DBD and LBD sections. The sequences of the hinge region vary significantly

among NRs and contribute to flexible rotations of the DBD, enabling direct or inverted binding and dimerization of NRs onto the HREs [20]. There is some evidence that this region may also serve as a binding site for co-repressor proteins [21,22].

The LBD has only moderate sequence conservation among NRs, but possess highly similar three-dimensional organizations. The LBD is composed of 11–12 alpha helices, which can fold into a compact formation with parallel and anti-parallel orientation (Fig. 3B). This domain is associated with multiple functions including ligand attachment through the ligand-binding site (LBS), interactions with other proteins at the AF-2 and binding function-3 (BF-3) co-regulator binding sites, protein dimerization, and nuclear translocation [23]. For more in-depth discussions on AF-2 and BF-3 sites in NRs, we refer readers to the two recent review papers by Buzon et al. [24] and Caboni and Lloyd [17].

In addition to high-throughput screening and other conventional approaches, computer-aided drug design, guided by available protein structures in the Protein Data Bank (PDB) [25] and synthetic compounds from databases such as ZINC [26] can provide a cost- and time-effective strategy to search for new drug



**Fig. 3.** Sequence conservation and a general 3D structural model of protein domains in nuclear receptors. (A) A heat map at the top panel illustrates a multiple protein sequence alignment among the 48 NRs at the DNA binding domain (DBD) and ligand binding domain (LBD), generated by using Clustal Omega [12] and visualized in Jalview [162]. The domain boundary positions are defined according to NCBI CDD [161]. A darker color represents a residue that matches the consensus sequence (more conserved) at a position, while a lighter color represents a different residue but with a positive Blosum62 score (less conserved). A non-conserved residue or a gap is colored in white. The 48 NRs are arranged from top to bottom (y-axis) in the same order as they appear in Table 1. The aligned residues are arranged from the N-terminus to the C-terminus on the x-axis. Residues within the LBS, AF-2 and BF-3 sites are highlighted as black bars on top of the LBD heat map, using the AR sequence as the reference positions defined by NCBI CDD [161] and Buzon et al. [24]. (B) The bottom panel shows a general 3D structural model, reconstructed by combining several crystal structures of individual domains, which include: (1) DBD (in pink ribbon) bound at DNA (PDB ID: 1R4I) [163]; (2) the relative orientation among DBD, the hinge region and LBD (PDB ID: 4IQR) [164]; (3) LBD (in orange ribbon) and the ligand testosterone bound at the ligand-binding site (PDB ID: 2AM9) [165]; (4) LBD with a small molecule inhibitor bound at the AF-2 site (PDB ID: 2YHD) [35]; and (5) LBD with a small molecule inhibitor bound at the BF-3 site (PDB ID: 4HLW) [37]. No structural model of the N-terminal regions is available. Protein models were visualized by using the Molecular Operating Environment (MOE).

candidates and has been successfully applied to a number of NRs [17,23]. For instance, virtual screening methods have been used to discover agonists and antagonists targeting the ligand binding domains of PPAR [27], RAR [28], estrogen related receptor (ERR) [29] and Liver receptor homolog 1 (LRH-1) [30]. While many existing drugs targeted the ligand binding site within the LBD of NRs, such as Enzalutamide [31] and Bicalutamide [32] for AR and Tamoxifen [33] and Fulvestrant [34] for ER, there are several new inhibitors that have been developed to target protein–protein interactions at alternative sites, as exemplified by AF-2 [35] and BF-3 [36,37] of the androgen receptor (Table 3). These alternative sites (depicted in Fig. 3B) are required for physical interactions

with protein co-factors; thus, binding of small molecules at such sites can block co-factor interactions and provide additional means for inhibiting NRs, particularly in cases where drug resistance has occurred due to mutations in the LBS. Furthermore, it has been shown recently as a proof of concept that the protein–DNA interface on the DBD of AR can be targeted by small molecules [38,39], and this work opens up a new strategy to inhibit different protein variants that may differ in LBD but share the same DBD within the AR or in other NR subfamilies.

To evaluate the “druggability” and the number of available structural templates of each NR, protein sequences were aligned with those with existing protein structures in PDB [25] by using

**Table 2**

A list of orphan nuclear receptors featured in this review paper.

Name	UniProt ID	Endogenous ligands	Interactors (BioGRID)	Protein structures (PDB ID)	LBS/AF-2/BF-3 vs. AR (sequence identity, similarity)
ROR $\alpha$	P35398	Cholesterol, cholesterol sulfate, hydroxycholesterol, ketocholesterol, epoxycholesterol, ruscogenin [64]	24 Interactors (ID: 112022)	1N83, 1S0X	6/27/17%, 24/64/25%
ROR $\beta$	Q92753	Stearic acid, retinoic acid [64]	14 Interactors (ID: 112023)	1K4W*, 1NQ7*, 1N4H*	6/27/17%, 24/64/25%
ROR $\gamma$	P51449	Hydroxycholesterol, ketocholesterol, epoxycholesterol [64]	3 Interactors (ID: 112024)	3B0W, 3KYT, 3L0J, 3L0L, 4NB6, 4NIE	12/27/25%, 29/64/33%
LXR $\alpha$	Q13133	Oxysterols [90]	37 Interactors (ID: 115373)	1UHL, 3IPQ, 3IPS, 3IPU	18/36/25%, 35/64/25%
LXR $\beta$	P55055	Oxysterols [90]	54 Interactors (ID: 113222)	1P8D, 1PQ6, 1PQ9, 1PQC, 1UPV, 1UPW, 3KFC, 3L0E, 4DK7, 4DK8	18/36/25%, 35/64/25%
COUP-TFI	P10589	Orphan	20 Interactors (ID: 112883)	2EBL	18/9/17%, 35/73/25%
COUP-TFII	P24468	Orphan	30 Interactors (ID: 112884)	3CJW	18/9/17%, 35/73/25%
ERR $\alpha$	P11474	Orphan	44 Interactors (ID: 108405)	1XB7, 2PJL, 3D24, 3K6P	35/45/25%, 76/91/33%
ERR $\beta$	O95718	Orphan	7 Interactors (ID: 108406)	1L01	35/45/25%, 71/91/42%
ERR $\gamma$	P62508	Orphan	24 Interactors (ID: 108407)	1KV6, 1TFC, 1VJB, 2E2R, 2EWP, 2GP7, 2GPO, 2GPP, 2GPU, 2GPV, 2P7A, 2P7G, 2P7Z, 2ZAS, 2ZBS, 2ZKC	35/45/25%, 71/91/33%
NGFIB	P22736	Orphan	84 Interactors (ID: 109407)	2QW4, 3V3E, 3V3Q, 4JGV, 4KZI, 4KZJ, 4KZM	12/9/33%, 47/45/33%
NURR1	P43354	Orphan	14 Interactors (ID: 110983)	1OVL	18/0/33%, 47/45/42%
NOR1	Q92570	Orphan	4 Interactors (ID: 113713)	1CIT*	18/0/33%, 47/45/42%
DAX1	P51843	Orphan	15 Interactors (ID: 106695)	3F5C**	6/27/17%, 41/100/33%

The lack of endogenous ligands in ONRs was confirmed by querying the IUPHAR/BPS database [236] in October 2014. BioGRID: the number of interactors queried in October 2014. PDB: available protein structures queried in July 2014. Protein structure: \*homologous protein structures with  $\geq 70\%$  sequence identity. \*\*homologous protein structures with  $<70\%$  sequence identity. Sequence similarity was calculated based on the Blosom62 matrix vs. AR sequences. Residue positions within each functional site, LBS ( $n = 17$ ), AF-2 ( $n = 11$ ) and BF-3 ( $n = 12$ ), were defined based on the AR protein, according to NCBI Conserved Domain Database (CDD) [161] and Buzon et al. [24].

a standard BLAST algorithm [40]. As highlighted in Fig. 1 and Table 2, the majority of NRs and ONRs have protein structural templates with sequence identity more than 70%, which is sufficient for homology modeling as a basis for virtual screening. The multiple sequence alignment as represented as a heat map in Fig. 3A illustrates that the positions of amino acids at LBS, AF-2 and BF-3 sites overlap with highly conserved regions within LBD. This is in accordance with previous reports [24] indicating that these functional sites are often conserved among many NRs including members of the ONR family [41–45] and as such may be targetable. To facilitate future drug design targeting the ONRs, Table 2 shows a list of ONRs featured in this review paper and their annotations including accession numbers in the UniProt database [46], endogenous ligands, the number of interactors listed in the BioGRID database [47], and available protein structures in PDB. To determine the extent of sequence conservation at the LBS and possible presence of AF-2 and BF-3, the corresponding residues of each ONR from the multiple sequence alignment were compared to those of the androgen receptor, and the sequence identity/similarity values were listed in Table 2. Interestingly, the AF-2 site of RORs, LXRs, COUP-TFs, ERRs and DAX1 share moderate to high sequence similarities with the AR (64%, 64%, 73%, 91% and 100% respectively), and previous studies have confirmed the presence of AF-2 in RORs and ERRs [48,49].

In the next sections, we focus on these ONRs as potential drug targets in prostate and breast cancers. For each ONR, we review its functions in normal physiological and cancer-related conditions. The druggability of each highlighted ONR and known small molecule ligands are also discussed. Table 3 summarizes a selected list of existing drugs and experimental compounds targeting each

ONR, while synthetic compounds targeting AR and ER are also included for reference. For the roles of other NRs and ONRs in different cancers, we refer readers to a list of representative articles summarized in Table 1.

### Targeting ONRs in prostate and breast cancers

Prostate cancer is one of the most common cancers in men, with an estimated 238,590 newly diagnosed cases and 29,720 deaths in 2013 in the United States alone [50]. As a result of improved screening procedures, prostate cancers are currently being diagnosed earlier, when the disease is localized to the prostate; if detected early, the treatment options of radical prostatectomy and/or radiation therapy are potentially curative. However, the main treatment options for advanced or metastatic prostate cancers are hormonal therapies.

Prostate cancer, like the non-malignant prostate, is dependent on androgens for proliferation and survival. Thus, hormonal therapies that aim to either reduce androgen levels (via surgical or chemical castration) or inhibit the AR protein (by small molecules) are used to slow tumor growth. Unfortunately, despite initial response, such treatments will eventually fail and the cancer recurs in an incurable, castration-resistant form [51,52]. This treatment resistance may be a result of several mechanisms including amplification of AR expression, mutations at the androgen-binding site of the AR and elevated production of AR splice variants [53]. Thus, the development of new therapeutics to combat metastatic castration-resistant prostate cancer remains a major task.

Breast cancer is the leading cause of cancer-related death in women worldwide [54]. In 2013 alone, there were an estimated



Table 3 (continued)

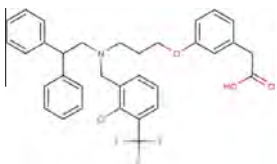
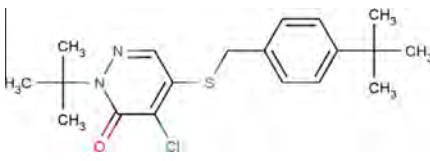
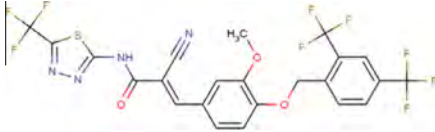
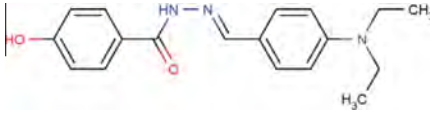
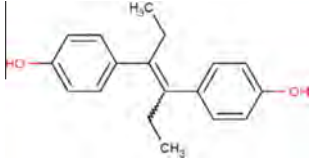
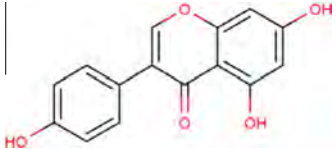
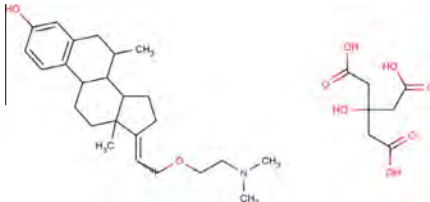
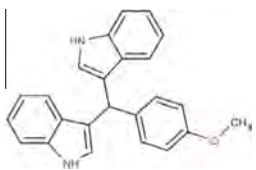
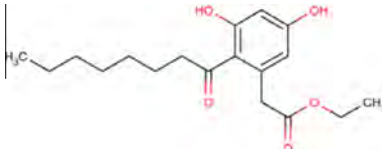
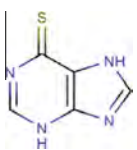
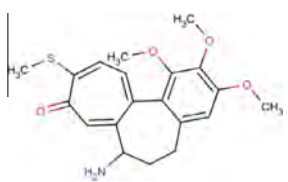
Compound name	Chemical structure	Annotation (protein target/accession number/status/activity)
GW3965		<ul style="list-style-type: none"> <li>- LXRs agonist [88]</li> <li>- CID 447905 (PubChem)</li> <li>- Experimental</li> <li>- EC<sub>50</sub> = 40 nM (FRET based cell-free ligand sensing assay), LXRB (PubChem BioAssay AID: 437600)</li> </ul>
Pyridaben		<ul style="list-style-type: none"> <li>- COUP-TFII antagonist (PubChem BioAssay AID: 720548)</li> <li>- CID 91754 (PubChem)</li> <li>- Experimental</li> <li>- IC<sub>50</sub> = 61.93 nM (luciferase reporter assay)</li> </ul>
XCT790		<ul style="list-style-type: none"> <li>- ERR<math>\alpha</math> inverse agonist [113]</li> <li>- CID 6918788 (PubChem)</li> <li>- Experimental</li> <li>- IC<sub>50</sub> = 0.37 <math>\mu</math>M (GAL4-ERR<math>\alpha</math> cell-based transfection assay) [130]</li> </ul>
DY131		<ul style="list-style-type: none"> <li>- ERR<math>\beta</math>/<math>\gamma</math> agonist [114,115]</li> <li>- CID 5497124 (PubChem)</li> <li>- Experimental</li> <li>- EC<sub>50</sub> = 0.13 <math>\mu</math>M (FRET assay), ERR<math>\gamma</math> (PubChem BioAssay AID: 246440)</li> </ul>
Diethylstilbestrol		<ul style="list-style-type: none"> <li>- ERR<math>\gamma</math> antagonist [128]</li> <li>- DB00255 (DrugBank)</li> <li>- Approved, menopausal/postmenopausal disorders</li> </ul>
Genistein		<ul style="list-style-type: none"> <li>- ERRs agonist [29]</li> <li>- CID 5280961 (PubChem)</li> <li>- Natural compound</li> </ul>
SR16388		<ul style="list-style-type: none"> <li>- ERR<math>\alpha</math> antagonist [131]</li> <li>- CID 54612678 (PubChem)</li> <li>- Experimental/Clinical trial</li> </ul>
DIM-C-pPhOCH <sub>3</sub> (C-DIM)		<ul style="list-style-type: none"> <li>- NGFIB agonist/antagonist [133]</li> <li>- CID 11371604 (PubChem)</li> <li>- Experimental</li> </ul>
Cytosporone B		<ul style="list-style-type: none"> <li>- NGFIB agonist [142]</li> <li>- CID 10687292 (PubChem)</li> <li>- Natural compound</li> <li>- EC<sub>50</sub> = 0.115 nM (luciferase reporter assay), PubChem, BioAssay AID: 500599</li> </ul>
6-Mercaptopurine		<ul style="list-style-type: none"> <li>- NGFIB-like agonist [148]</li> <li>- DB01033 (DrugBank)</li> <li>- Approved, antineoplastic agent</li> </ul>

Table 3 (continued)

Compound name	Chemical structure	Annotation (protein target/accession number/status/activity)
Thiocolciran		<ul style="list-style-type: none"> <li>- DAX1 antagonist (PubChem BioAssay AID: 687017)</li> <li>- CID 426728 (PubChem)</li> <li>- Experimental</li> <li>- IC<sub>50</sub> = 212.03 nM (luciferase reporter assay)</li> </ul>

Accession numbers are provided for either DrugBank [6] or PubChem [224], and chemical structures were visualized by using MarvinSketch.

232,340 newly diagnosed cases and 39,620 deaths in the United States [50]. Based on the gene expression profiles, breast cancers can be divided into five subtypes that vary in their treatment options and survival outcomes [55–58]. ER $\alpha$  positive (ER $\alpha$ +) and progesterone receptor positive (PR+) tumors account for approximately 70% of all cases [59,60]. Additionally, based on the expression or gene amplification of the ErbB-2/HER2 gene, these ER $\alpha$ +/PR+ tumors can be further classified into HER2- and HER2+ subtypes. For both early and advanced ER $\alpha$ +/PR+ breast cancer, the standard treatment is to block the production of estrogens (e.g. aromatase inhibitors) or estrogen binding to the receptor (e.g. Tamoxifen). Adjuvant Trastuzumab therapy, which specifically targets the HER2 receptor, is used to treat ER $\alpha$ -/PR-/HER2+ tumors and has resulted in positive survival outcomes. In contrast, the ER $\alpha$ -/PR-/HER2- subtype or triple negative breast cancer, which accounts for 10–17% of all breast cancer cases, and the normal breast-like (basal-like) cancer subtype, which accounts for 15% of the cases, are both without approved targeted therapies [61]. Unfortunately, drug resistance to even the most effective therapeutics is a major issue affecting breast cancer patients today. For example, although initially effective, one third of patients treated with Tamoxifen develop recurrent diseases within 15 years due to the progression of surviving tumor cells to a hormone-resistant state [62]. For these reasons, alternative drug treatments, which can be used either alone or in combination with existing therapies, are needed.

#### RAR-related orphan receptors (RORs), NR1F

The retinoic acid-receptor-related orphan receptor (ROR) family consists of three members: ROR $\alpha$  (NR1F1), ROR $\beta$  (NR1F2), and ROR $\gamma$  (NR1F3). RORs have been shown to regulate lipid and steroid metabolism, immune response and circadian rhythms. ROR $\alpha$  is widely expressed in many tissues. ROR $\beta$  is expressed in the central nervous system in particular it is localized to regions that process sensory information, such as the retina and pineal gland [63,64]. ROR $\gamma$  is also broadly expressed but in particular it is expressed in immune tissues such as the thymus, and is a significant factor in the development of T helper 17 cells [65]. A number of putative natural and synthetic ligands have been identified that target RORs; most notably cholesterol, cholesterol metabolites and oxysterols that have been shown to bind to the LBD of RORs and induce conformational changes that affect coregulator interactions [66,67]. While recent research has focused on the therapeutic value of inhibiting RORs for treating metabolic and autoimmune diseases, analyses of published gene expression data indicate that ROR $\alpha$  is downregulated in multiple cancers including prostate, breast and pancreatic cancer, and suggest that ROR $\alpha$  may act as a tumor suppressor [66,68–70].

In prostate cancer, the role of ROR $\alpha$  as a tumor suppressor was demonstrated *in vitro* and *in vivo* using the thiazolidinedione-derivative CGP 52608 (Pubchem CID: 6509863, Table 3) [69,70]. The activation of ROR $\alpha$  by CGP 52608 resulted in reduced cell

proliferation, migration and invasiveness *in vitro*, as well as reduced growth of xenograft tumors in nude mice using the androgen-independent prostate cancer cell line, DU145 [69,70]. In addition, these studies demonstrated that activation of ROR $\alpha$  leads to an increased expression of the cyclin-dependent kinase inhibitor p21, but decreases the expression of cyclin A. These findings, combined with the observations that there are response elements for ROR $\alpha$  at promoter regions upstream of several cell cycle-related genes [69], suggests that ROR $\alpha$  plays an inhibitory role in cell cycle regulation. In addition, it has recently been shown that activation of ROR $\alpha$ , as well as ROR $\gamma$ , by the agonist SR1078 (Pubchem CID: 17980288, Table 3) can stabilize p53 protein a well-known tumor suppressor; an observation that further substantiates a tumor suppressing role of ROR $\alpha$  [10,71,72]. However, further investigation is required to determine if activation of reduced protein levels of ROR $\alpha$  in cancer would be enough to initiate adequate tumour suppression.

In breast cancer, several lines of evidence also support the role of ROR $\alpha$  as a tumor suppressor. ROR $\alpha$  expression is downregulated in breast cancer tumor cells compared to normal mammary tissue [73,74]. Inhibition of ROR $\alpha$  expression results in the disruption of polarized acinar cells of mammary gland, and this disruption is known to be an early event in breast cancer development [75]. In contrast, overexpression of ROR $\alpha$  in MCF12F breast cancer cells resulted in a reduced rate of cell proliferation [73]. ROR $\alpha$  has been shown to act upon several pathways that are known to be involved in breast cancer development and progression. ROR $\alpha$  binds to  $\beta$ -catenin that suppresses Wnt/ $\beta$ -catenin gene transcription, which is known to be an important pathway in cell adhesion and cell growth [76,77]. In addition, ROR $\alpha$  has been shown to have a negative effect on the NF- $\kappa$ B pathway, which is known to regulate genes important in cell proliferation, differentiation and apoptosis in breast cancer [78–81]. Additionally, ROR $\alpha$  plays a role in steroidogenesis. In ROR $\alpha$  knockout mice, the regulation of hydroxysteroid dehydrogenases & sulfotransferases were disrupted [82]. Furthermore, ROR $\alpha$  has been shown to enhance the expression of aromatase in ER $\alpha$ + T47D and MCF7 breast cancer cells through its binding to the aromatase promoter [83].

In addition to the discovery of compounds CGP 52608 and SR1078, there have been efforts to screen for ROR $\alpha$  inhibitors at the Scripps Research Institute Molecular Screening Center (PubChem BioAssay AID: 610). They reported on several inhibitory compounds with IC<sub>50</sub> values of <5 nM in a transcriptional activity assay (AC1M6FQE, Table 3). There are currently over 590 experimental compounds in the BindingDB that target the ROR $\alpha$ , although none of them have been clinically approved (Fig. 1) [7]. Based on the existing knowledge on natural and synthetic ROR ligands (reviewed by Kojetin and Burris [64]) and considering the availability of a crystal structure of the ligand binding domain of ROR $\alpha$  in complex with cholesterol (PDB ID: 1N83) [84], structural analyses of the LBS and virtual compound screening could be used to discover novel ROR $\alpha$  agonists or antagonists and to improve the drug-like properties of existing candidates.

### Liver X receptors (LXRs), NR1H

As the name suggests, liver X receptors (LXRs) were initially isolated from liver, but subsequent studies have shown that while the expression of LXR $\alpha$  is more restricted to certain tissues, such as the liver, kidney, and adipose, its  $\beta$  isoform is ubiquitously expressed in almost all tissue types (Table 1) [85]. The main functional role of LXRs is to regulate lipid homeostasis, and activation of LXRs leads to the reduction of intracellular cholesterol [86]. Since cholesterol is a precursor needed for androgen synthesis, activating LXRs can reduce androgen levels in prostate cancer cells [86].

It has been reported that a synthetic LXR agonist T0901317 (Pubchem CID: 447912, Table 3), which mimics the structure of the endogenous ligand oxysterol, was able to decrease cell proliferation of the androgen-dependent LNCaP prostate cancer cell line, as well as suppress the growth of LNCaP tumor xenografts in nude mice [85,87]. This anti-proliferative effect of the synthetic LXR agonist was deemed to be a result of increased expression of cyclin-dependent kinase inhibitor p27 and a decreased cell population in S-phase [87]. In addition, a recent study has reported that activation of LXR by agonist GW3965 (Pubchem CID: 447905, Table 3) reduced cellular proliferation and migration in LNCaP cells and reduced tumor volumes in LNCaP-xenografted nude mice, possibly through regulation of the suppressor of cytokine signaling 3 (SOCS3) pathway [88].

It should be noted, that LXRs are both anti-proliferative and lipogenic factors in breast cancer. By using a microarray gene analysis of four breast cancer cell lines, it was shown that LXR ligands disrupt cell proliferation by down-regulating genes in the E2F transcription factor family that are critical for cell cycle progression [89]. Additionally, LXR induces the expression of key regulatory genes, such as fatty acid synthase, leading to increased triglyceride production within MCF7 breast cancer cells [90]. Furthermore, targeting LXR also stimulates the efflux of cholesterol within the cell which leads to the inhibition of cell proliferation and stimulation of apoptosis in MCF7 cells [91,92]. Although the full functionality of LXRs and their ligands are yet to be fully understood, with the available evidence, LXRs could be a promising target for the treatment of breast cancer.

LXRs are one of the most challenging members of the NR family to target on account of their essential role in hepatic lipogenesis. As a consequence there is an additional layer of complexity to the rational drug design in that the therapeutics must be tissue selective to reduce side effects. There are no approved substances reported in the DrugBank [6] despite the fact that there are over 700 experimental LXR ligands deposited in the BindingDB [7]. A number of crystal structures of the LXR  $\alpha$  and  $\beta$  forms have been resolved, including LXR $\beta$  in complex with an agonist T0901317 (PDB ID: 1UPW) [93]. Both T0901317 and GW3965 are agonists for both LXR $\alpha$  and LXR $\beta$ . While the development of selective activators for LXR $\alpha$  and LXR $\beta$  has been difficult due to their highly similar amino acid sequence (74%), several recent studies have described compounds that display partial selectivity to LXR $\beta$  [94–97]. Overall, although challenging, the abundance of existing compounds and crystal structures should facilitate both ligand- and structure-based rational drug design efforts against LXR $\alpha$  and LXR $\beta$ .

### Chicken ovalbumin upstream promoter transcription factors (COUP-TFs), NR2F

Currently, chicken ovalbumin upstream promoter transcription factor (COUP-TF) I and II, (reviewed by Lin et al. [98]) have no endogenous ligands identified. COUP-TFI and II are known to have important roles in embryonic and organ development [99]. COUP-TFs bind to DNA repeats as homo- or heterodimers [100,101].

COUP-TFs interact with the same ER $\alpha$  and ER $\beta$  DNA response elements, the estrogen response element (ERE); however, COUP-TFs bind to half EREs whereas ERs preferentially bind to the consensus EREs [102]. Klinge et al. demonstrated that COUP-TFs are able to directly interact with and disturb ER-DNA binding and as a consequence regulate the transcriptional activity of ER $\alpha$  [103]. Although the full relationship with ER is still unclear as a study by Metivier et al. demonstrated that the formation of a COUP-TF/ER $\alpha$  complex resulted in enhanced ER $\alpha$  transcriptional activity [104].

In the prostate, COUP-TFs may adopt either a tumor-promoting or tumor-suppressing role, depending on the cellular context and surrounding tumor microenvironment [99]. On one hand, it has been shown that COUP-TFII can promote prostate tumor growth by inhibiting the TGF- $\beta$ -induced growth barrier and that overexpression of COUP-TFII can promote tumor metastasis when coupled with PTEN deletion [105]. On the other hand, another study reported that COUP-TFII can repress AR activation by binding to both the DBD and LBD of the AR and that overexpression of COUP-TFII reduces proliferation of LNCaP cells [106]. In addition, recent genome-wide gene expression analysis and transcription factor binding experiments revealed that COUP-TFI is a direct target gene of the AR and that its expression is downregulated upon AR binding at the promoter region [107]. This study further reported an inverse relationship between COUP-TFI gene expression and AR activation in a normal prostate development phase.

While the exact function of COUP-TFII in breast cancer remains unclear, its mRNA expression in ER $\alpha$ + (MCF7, T47D, and ZR75-1) and ER $\alpha$ - (MDA-MB-231) breast cancer cells was shown to be significantly increased in comparison to normal epithelial cell lines [108]. Interestingly, COUP-TFII levels were reduced in Tamoxifen-resistant lines derived from MCF7 cells, and re-expression of COUP-TFII restored the growth inhibitory effect of Tamoxifen [104,109]. Further to this study, Litchfield et al. suggested that reduced expression of COUP-TFII in the endocrine resistant cell line LCC9 contributed resistance due to the suppression of NF $\kappa$ B activity [110].

To date, there are a number of compounds reported for COUP-TFII (but not for COUP-TFI) and no approved drugs that target the COUP-TF family. There are 231 experimental compounds with reported binding affinities toward COUP-TFII in the BindingDB deposit [7]. Among these, pyridaben (PubChem CID: 91754, Table 3) has an IC<sub>50</sub> of 62 nM that was determined in a transcriptional assay by the Scripps Research Institute Molecular Screening Center (PubChem BioAssay AID: 720548). The crystal structure of the ligand binding domain of COUP-TFII is available (PDB ID: 3CJW) and shows that  $\alpha$ -helix 10 extends into the LBD and that the AF-2 is folded into a position that blocks coregulator interactions resulting in autorepression of the receptor [111]. However, the receptor still contains a viable ligand binding pocket that when bound with ligand releases the autorepression [111]. Due to a high sequence similarity (96%) at LBD, the COUP-TFII structure can be used as a template for COUP-TFI for further *in silico* screening. In particular, *in silico* screening should be applied to discover chemical structures with high binding affinity at the LBS of COUP-TFs. Such compounds may act as either agonists or antagonists and can be used as chemical probes to further elucidate the functions of COUP-TFs in prostate and breast cancer cells.

### Estrogen related receptors (ERRs), NR3B

The estrogen-related receptors (ERRs), ERR $\alpha$ , ERR $\beta$  and ERR $\gamma$ , were so named for their sequence and structural similarity to estrogen receptors (ERs) [112]. While initially thought to share a common biological function with the ERs, ERRs do not bind to estrogens or endogenous ER ligands. There are no known endogenous ligands for ERRs, and the receptors are constitutively active

due to the structure of the LBD that leaves the AF-2 site exposed and capable of binding coregulators in the absence of ligand binding. Thus, the transcriptional properties of the ERRs are mediated by either: coactivators, such as peroxisome-proliferator activated receptor  $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) and the p160 family of steroid receptor coactivators (SRC-1, SRC-2, SRC-3), or corepressors such as nuclear receptor interacting protein 140 (RIP140). The active ERR is able to bind to DNA at the 5'-TNAAGGTCA-3' sequence (where N is any nucleotide) or to the classical ER response element (ERE) 5'-AGGTCANNNTGACCT-3'. ERRs regulate genes involved in the cellular energy metabolism pathways, including: glycolysis, tricarboxylic acid cycle and oxidative phosphorylation [112]. Thus, ERR $\alpha$  is predominantly expressed in tissues that have high energy demand, including skeletal muscle, heart, kidney, liver and adipose. Whereas, ERR $\beta$  is expressed during early embryonic development and ERR $\gamma$  is expressed in the central nervous system and spinal cord.

Of the ERRs, ERR $\alpha$  is known to promote cell proliferation in multiple cancer cells lines, including prostate and breast cancer cells. Furthermore, inhibition of ERR $\alpha$  with the inverse agonist (i.e. binds to the same receptor protein but induces an opposite response to an agonist) XCT790 (Pubchem CID: 6918788, Table 3) reduces cell proliferation [113]. However, overexpression of either ERR $\beta$  or ERR $\gamma$  resulted in the suppression of cell proliferation in both androgen-sensitive and androgen-insensitive prostate cancer cells, and this growth inhibition could be further enhanced by the ERR $\beta/\gamma$  agonist, DY131 (Pubchem CID: 5497124, Table 3) [114–116]. In addition, transcription reporter and chromatin immunoprecipitation assays demonstrated that ERR $\beta$  directly transactivates a promoter upstream of the cyclin-dependent kinase inhibitor p21 gene, which inhibits cell cycle progression [115]. While the mechanism through which the three ERR isoforms induce their effects on cell proliferation is undetermined, these findings suggest that in prostate cancer, ERR $\alpha$  acts as a tumor promoter, whereas ERR $\beta$  and ERR $\gamma$  act as tumor suppressors.

Due to their structural similarity to ERs, ERRs (in particular ERR $\alpha$  and ERR $\gamma$ ) are most recognized for their potential function in breast cancer. Members of the ERR family can bind to EREs and affect gene expression of ER-regulated genes and may contribute to breast cancer development or progression. Interestingly, Stein et al. showed that the gene transcription products of ERR $\alpha$  had little overlap with those of ER [117]. Furthermore, the roles of both ERR $\alpha$  and ERR $\gamma$  are distinct between ER $\alpha$ + and ER $\alpha$ - breast cancer. ERR $\alpha$  can act as an activator or repressor of transcription in ER $\alpha$ - or ER $\alpha$ + cells, respectively [118]. For example, ERR $\alpha$  can increase the expression of the estrogen-regulated trefoil factor 1 (TFF1) gene in ER $\alpha$ - breast tumors [119,120]. Similarly, in ER $\alpha$ - breast cancer samples, overexpression of ERR $\alpha$  induced vascular endothelial growth factor (VEGF) [117,121]. In comparison, ERR $\gamma$  is predominantly associated with ER $\alpha$ +/PR+ breast tumors in which it promotes mesenchymal-to-epithelial transition (MET) by stimulating E-cadherin expression, which results in an inhibition of tumor growth [122,123]. Furthermore, ERR $\gamma$  expression may be a marker of tamoxifen resistance and it has been shown that an AAAG tetranucleotide repeat polymorphism in untranslated region of the ERR $\gamma$  gene is associated with breast cancer susceptibility [124,125]. While the exact mechanism of action of both ERR $\alpha$  & ERR $\gamma$  in breast cancer is still not fully understood, current findings support a potential role of these receptors as therapeutic targets in breast cancer.

There are relatively few compounds reported to interact with ERRs (reviewed Lu et al. [126]). There are several crystal structures resolved for the ligand binding domains of ERR $\alpha$  and ERR $\gamma$ . No crystal structure is available for the LBD of ERR $\beta$ , but the high protein sequence identity (80%) with ERR $\gamma$  makes homology modeling feasible. However, the small size of the ligand binding pocket has

made it difficult to directly target ERRs for pharmacologic activation [127]. Hence, the BindingDB catalogues only 91, 31, and 29 compounds that can bind to ERR $\alpha$ ,  $\beta$  and  $\gamma$ , respectively [7]. The FDA-approved drugs diethylstilbestrol (DB00255, Table 3) and tamoxifen have been shown to interact with ERRs and act as antagonists [128,129]. Whereas, XCT790 (Pubchem CID: 6918788, Table 3) binds selectively to the LBD of ERR $\alpha$  and has an IC<sub>50</sub> of 0.37  $\mu$ M [130]. Methods that combined structure-based virtual screening and biological functional assays have identified several isoflavones including Genistein that bind at the LBS of the ERRs [29]. SR16388 (Pubchem CID: 54612678, Table 3), a novel steroidal antiestrogen, disrupts the interaction between ERR $\alpha$  and its coactivator peroxisome-proliferator activated receptor  $\gamma$  coactivator-1 $\alpha$  to inhibit ERR $\alpha$  activity. SR16388 has an IC<sub>50</sub> of 0.2  $\mu$ M on malignant cell growth in xenograft models and has successfully completed phase I and phase II clinical trials [131].

#### *Nerve growth factor 1B-like (NGFIB, NURR1, NOR1), NR4A*

The nerve growth factor 1B-like family is comprised of three members: NGFIB (NUR77 or NR4A1), NURR1 (NR4A2), and NOR1 (NR4A3). These nuclear receptors are unique due to the fact that they lack the LBS within their LBD [45]. Members of this family regulate a wide range of biological processes including metabolism, inflammation, vascular function, steroidogenesis, and functions of the central nervous system [132,133]. NGFIB is known to interact with specific response elements 5'-AAAGGTCA-3' in the form of a monomer or homodimer [134,135]. The physiological role of NGFIB is not yet fully understood, but several studies indicate it has a role in cancer cell death and survival [136–139]. Interestingly, NGFIB is found to have pro-survival benefits while in the nucleus, but promote apoptosis through Bcl-2/cytochrome c pathway when translocated into mitochondria [140,141]. NGFIB has been previously shown to exhibit pro-oncogenic activity in several cancers, including cervical, lung, colon and pancreatic cancers. A number of compounds, including 1,1-bis(3'-indolyl-1-(p-substituted phenyl)methanes (C-DIM; Pubchem CID: 54612678, Table 3), which can activate or deactivate NGFIB, have been reviewed [133]. In addition, cytosporone B has been previously reported as a naturally occurring agonist for NGFIB and was capable of promoting apoptosis and reducing xenograft tumor growth by inducing NGFIB expression and translocation to mitochondria [142].

With regard to prostate cancer, a recently reported immunohistochemistry experiment revealed that expression of NURR1 protein is higher in prostate cancer tissues compared to benign ones [143]. Furthermore, this NURR1 expression was positively correlated with several clinical indicators, including tumor stage and Gleason score. Knockdown of NURR1 protein with siRNA resulted in reduced proliferation, migration and invasion as well as induced apoptosis in prostate cancer cells. Hence, the study suggested that NURR1 may be used as a biomarker and therapeutic target in prostate cancer [143].

In breast cancer, various diindolylmethanes (DIMs) have demonstrated agonistic effects toward the NGFIB [144]. Chinthalapalli et al. demonstrated that in the presence of DIM, MCF7 breast cancer cells that express NGFIB have a reduction in their proliferation capacity and increased apoptosis [145]. In addition, NGFIB is known to regulate and affect transcriptional responses in other ONRs and NRs, such as COUP-TFs and retinoid X receptors (RXRs). Furthermore, it has been demonstrated that NGFIB is capable of producing a heterodimer with RXR that induces apoptosis and growth inhibition in MDA-MB-231 breast cancer cells through activation of RAR $\alpha$  and subsequent pathways [146].

There is not yet an approved drug targeting any member of the NR4A subfamily. The recent research efforts dedicated toward the NGFIB have generated 281 experimental compounds targeting this

protein. There are currently two crystal structures available for NGFIB (PDB ID: 2QW4 and 3V3E [147]), which should further aid in the identification or modification of new or existing compounds. In contrast, there are only 12 compounds which have been shown to interact with NURR1. The crystal structure of the NURR1 LBD (PDB ID: 1OVL [45]) is observed to be filled by hydrophobic substituents, which suggests that it may be a true ONR without a ligand [45]. Interestingly, 6-mercaptopurine and a few structural analogues have been shown to activate the NR4A subfamily. The action of 6-mercaptopurine occurs through the NTD in an AF-1 dependent manner [148]. The NTD of NRs is traditionally thought of as being structurally unstable and thus has not been the focus of many drug targeting approaches. However, given the identification of NTD directed compounds, further work in this direction may yield more promising results that could be applied to other ONR and NR targets.

#### DAX1, NROB1

While the physiological significance of DAX1 in females is unclear, knockout mouse studies have demonstrated that DAX1 is essential for the maintenance of male reproductive functions [149]. Furthermore, DAX1 is uniquely and highly expressed in steroidogenic tissues [150]. For example, DAX1 is known to be a negative regulator of aromatase mRNA expression in ovarian cells that are the sites of androgen and estrogen production [151]. Additionally, several studies suggest that the primary function of DAX1 is as a coregulator protein, since it is capable of affecting the activity of several receptors, including an ONR, steroidogenic factor-1 (SF-1), as well as, ER, PR and AR [152].

While not detected in normal prostate tissue, DAX1 is expressed in prostate cancer epithelium, and its expression is inversely correlated with Gleason score [153]. DAX1 has been shown to directly interact with the AR, inhibiting its transcriptional activity through disruption of the AR N- and C-terminal interaction [154]. DAX1, which is able to cycle between the cytoplasm and the nucleus, is also capable of tethering the AR in the cytoplasm or nucleus of the cell, further restricting AR transcriptional activity [154].

A recent publication by Conde et al. established that while DAX1 is expressed in the benign form of breast carcinoma (*in situ*), it is more significantly expressed in invasive breast cancer [155]. There was also a report of a direct correlation between lymph node metastasis and the expression of DAX1 protein. In addition, it was observed that DAX1 has also been shown to directly interact with AR, ER $\alpha$  and ER $\beta$  in breast cells, but has only been shown to influence the intracellular positioning of AR and ER- $\beta$ , and not ER- $\alpha$  [155,156]. Thus, DAX1 may be a novel coregulator of nuclear receptor action in both prostate and breast tissues.

DAX1 is unique among other members of the NR family in that it lacks a classical DBD domain and it has an N-terminal domain that contains three repeats of a unique 70 amino acid motif [157]. Additionally, DAX1 contains a potent transcriptional repressor domain in its C-terminus [158,159]. Although there is not yet a protein structure available for human DAX1, the LBD of the mouse homolog has been resolved (PDB ID: 3F5C [160]) and established that the C-terminal domain does not contain a ligand binding site. The structure of the human domain shares 68% identity with the mouse so it is possible that the human DAX1 LBD surface may contain a pocket that could be targeted by small molecules. Additionally, drugs may also be developed that target alternative surfaces of DAX1, such as areas involved in coregulator interactions or the N-terminal domain because of its unique sequence repeat. Currently, there are over 200 compounds indicated in BindingDB to bind to DAX1 [7]. The Scripps Research Institute Molecular Screening Center (PubChem BioAssay AID: 687017) reported that compound thicolciran (Pubchem CID: 426728, Table 3) demonstrates an IC<sub>50</sub> of

212 nM in a luminescence-based high-throughput cell screening assay. Additionally, there are two FDA-approved drugs that have been shown to indirectly target DAX1 [6]. One is dexamethasone, a potent glucocorticoid, and the other is Tretinoin, a naturally occurring derivative of vitamin A. Thus, DAX1 is a promising target due to its unique structure that may provide an alternative strategy for drug targeting.

#### Conclusions

In recent decades nuclear receptors have been actively exploited as drug targets mainly due to their regulatory roles in numerous cellular processes and the presence of “druggable” ligand binding domain. Unlike most other transcriptional factors that lack a well-defined active site, the function of NRs can be modulated by small molecules that compete with endogenous ligands to act as either antagonists or agonists. Within the NR family, hormone receptors such as the AR and ER have been studied intensively, and represent established targets for a number of approved drugs in prostate and breast cancers. However for both cancers, current hormonal therapies often lead to the development of drug resistance; thus, targeting alternative proteins and pathways may provide a way to treat resistant diseases. Given the recent advances in computational modeling techniques and the availability of protein structures, ONRs present an exciting opportunity for drug development. As described in this review, with the aid of *in silico* modeling much progress has been made on the identification of natural and synthetic ONR ligands. Furthermore the recent successes targeting alternative protein–protein interaction sites such as the AF-1, AF-2, and BF-3 site of the AR highlight the possibilities for targeting these sites on ONRs. Ultimately, identification of novel small molecules that bind to ONRs will provide a better understanding of their biology, structure and interactions. In addition, this accumulated knowledge on the endogenous functions of ONRs will provide a solid foundation for future drug discovery with therapeutic values that may go beyond prostate and breast cancers.

#### Conflict of interest

Authors declare no conflict of interest.

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#### References

- [1] Giguere V. Orphan nuclear receptors: from gene to function. *Endocr Rev* 1999;20:689–725.
- [2] Shi Y. Orphan nuclear receptors in drug discovery. *Drug Discov Today* 2007;12:440–5.
- [3] Nuclear Receptors Nomenclature Committee. A unified nomenclature system for the nuclear receptor superfamily. *Cell* 1999;97:161–3.
- [4] Darnell Jr JE. Transcription factors as targets for cancer therapy. *Nat Rev Cancer* 2002;2:740–9.
- [5] Overington JP, Al-Lazikani B, Hopkins AL. How many drug targets are there? *Nat Rev Drug Discovery* 2006;5:993–6.
- [6] Knox C, Law V, Jewison T, Liu P, Ly S, Frolkis A, et al. DrugBank 3.0: a comprehensive resource for ‘omics’ research on drugs. *Nucleic Acids Res* 2011;39:D1035–41.
- [7] Liu T, Lin Y, Wen X, Jorissen RN, Gilson MK. BindingDB: a web-accessible database of experimentally determined protein–ligand binding affinities. *Nucleic Acids Res* 2007;35:D198–201.

- [8] Massie CE, Lynch A, Ramos-Montoya A, Boren J, Stark R, Fazli L, et al. The androgen receptor fuels prostate cancer by regulating central metabolism and biosynthesis. *EMBO J* 2011;30:2719–33.
- [9] Bentzon N, Düring M, Rasmussen BB, Mouridsen H, Kroman M. Prognostic effect of estrogen receptor status across age in primary breast cancer. *Int J Cancer* 2008;122:1089–94.
- [10] Baek SH, Kim KI. Emerging roles of orphan nuclear receptors in cancer. *Annu Rev Physiol* 2014;76:177–95.
- [11] Huang P, Chandra V, Rastinejad F. Retinoic acid actions through mammalian nuclear receptors. *Chem Rev* 2014;114:233–54.
- [12] Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 2011;7:539.
- [13] Warnmark A, Treuter E, Wright AP, Gustafsson JA. Activation functions 1 and 2 of nuclear receptors: molecular strategies for transcriptional activation. *Mol Endocrinol* 2003;17:1901–9.
- [14] Tora L, Gronemeyer H, Turcotte B, Gaub MP, Chambon P. The N-terminal region of the chicken progesterone receptor specifies target gene activation. *Nature* 1988;333:185–8.
- [15] Chambon P. A decade of molecular biology of retinoic acid receptors. *FASEB J* 1996;10:940–54.
- [16] Giguere V. Retinoic acid receptors and cellular retinoid binding proteins: complex interplay in retinoid signaling. *Endocr Rev* 1994;15:61–79.
- [17] Caboni L, Lloyd DG. Beyond the ligand-binding pocket: targeting alternate sites in nuclear receptors. *Med Res Rev* 2013;33:1081–118.
- [18] Guo W, Burris TP, Zhang YH, Huang BL, Mason J, Copeland KC, et al. Genomic sequence of the DAX1 gene: an orphan nuclear receptor responsible for X-linked adrenal hypoplasia congenital and hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 1996;81:2481–6.
- [19] Seol W, Choi HS, Moore DD. An orphan nuclear hormone receptor that lacks a DNA binding domain and heterodimerizes with other receptors. *Science* (New York, NY) 1996;272:1336–9.
- [20] Glass CK. Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers. *Endocr Rev* 1994;15:391–407.
- [21] Chen JD, Evans RM. A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature* 1995;377:454–7.
- [22] Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, et al. Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* 1995;377:397–404.
- [23] Lallous N, Dalal K, Cherkasov A, Rennie PS. Targeting alternative sites on the androgen receptor to treat castration-resistant prostate cancer. *Int J Mol Sci* 2013;14:12496–519.
- [24] Buzon V, Carbo LR, Estruch SB, Fletterick RJ, Estebanez-Perpina E. A conserved surface on the ligand binding domain of nuclear receptors for allosteric control. *Mol Cell Endocrinol* 2012;348:394–402.
- [25] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The protein data bank. *Nucleic Acids Res* 2000;28:235–42.
- [26] Irwin JJ, Sterling T, Mysinger MM, Bolstad ES, Coleman RG. ZINC: a free tool to discover chemistry for biology. *J Chem Inform Model* 2012;52:1757–68.
- [27] Lewis SN, Bassaganya-Riera J, Bevan DR. Virtual screening as a technique for PPAR modulator discovery. *PPAR Res* 2010;2010:861238.
- [28] Schapira M, Raaka BM, Samuels HH, Abagyan R. Rational discovery of novel nuclear hormone receptor antagonists. *Proc Natl Acad Sci USA* 2000;97:1008–13.
- [29] Suetsugu M, Su L, Karlsberg K, Yuan YC, Chen S. Flavone and isoflavone phytoestrogens are agonists of estrogen-related receptors. *Mol Cancer Res* 2003;1:981–91.
- [30] Benod C, Carlsson J, Uthayaruban R, Hwang P, Irwin JJ, Doak AK, et al. Structure-based discovery of antagonists of nuclear receptor LRH-1. *J Biol Chem* 2013;288:19830–44.
- [31] Jung ME, Ouk S, Yoo D, Sawyers CL, Chen C, Tran C, et al. Structure-activity relationship for thiohydantoin androgen receptor antagonists for castration-resistant prostate cancer (CRPC). *J Med Chem* 2010;53:2779–96.
- [32] Schellhammer PF. An evaluation of bicalutamide in the treatment of prostate cancer. *Expert Opin Pharmacother* 2002;3:1313–28.
- [33] Jordan VC. Tamoxifen (ICI46,474) as a targeted therapy to treat and prevent breast cancer. *Br J Pharmacol* 2006;147(Suppl. 1):S269–76.
- [34] Curran M, Wiseman L. Fulvestrant. *Drugs* 2001;61:807–13 [discussion 14].
- [35] Axerio-Cilies P, Lack NA, Nayana MR, Chan KH, Yeung A, Leblanc E, et al. Inhibitors of androgen receptor activation function-2 (AF2) site identified through virtual screening. *J Med Chem* 2011;54:6197–205.
- [36] Ban F, Leblanc E, Li H, Munuganti RS, Frewin K, Rennie PS, et al. Discovery of 1H-indole-2-carboxamides as novel inhibitors of the androgen receptor binding function 3 (BF3). *J Med Chem* 2014;57:6867–72.
- [37] Munuganti RS, Leblanc E, Axerio-Cilies P, Labriere C, Frewin K, Singh K, et al. Targeting the binding function 3 (BF3) site of the androgen receptor through virtual screening. 2. Development of 2-((2-phenoxyethyl)thio)-1H-benzimidazole derivatives. *J Med Chem* 2013;56:1136–48.
- [38] Li H, Ban F, Dalal K, Leblanc E, Frewin K, Ma D, et al. Discovery of small-molecule inhibitors selectively targeting the DNA-binding domain of the human androgen receptor. *J Med Chem* 2014;57:6458–67.
- [39] Dalal K, Roshan-Moniri M, Sharma A, Li H, Ban F, Hessein M, et al. Selectively targeting the DNA-binding domain of the androgen receptor as a prospective therapy for prostate cancer. *J Biol Chem* 2014;289:26417–29.
- [40] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol* 1990;215:403–10.
- [41] Downes M, Verdecia MA, Roecker AJ, Hughes R, Hogenesch JB, Kast-Woelbern HR, et al. A chemical, genetic, and structural analysis of the nuclear bile acid receptor FXR. *Mol Cell* 2003;11:1079–92.
- [42] le Maire A, Teysier C, Erb C, Grimaldi M, Alvarez S, de Lera AR, et al. A unique secondary-structure switch controls constitutive gene repression by retinoic acid receptor. *Nat Struct Mol Biol* 2010;17:801–7.
- [43] Molnar F SR, Sato Y, Antony P, Mourino A, Rochel N, Moras D. Structural basis of the activation of the human vitamin d receptor by the natural metabolite 1alpha,25-dihydroxy-3-epi-vitamin d3. 2009/9/18 ed: NCBI Structure Summary MMDB; 2009.
- [44] Nolte RT, Wisely GB, Westin S, Cobb JE, Lambert MH, Kurokawa R, et al. Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-gamma. *Nature* 1998;395:137–43.
- [45] Wang Z, Benoit G, Liu J, Prasad S, Aarnisalo P, Liu X, et al. Structure and function of Nurr1 identifies a class of ligand-independent nuclear receptors. *Nature* 2003;423:555–60.
- [46] Activities at the universal protein resource UniProt. *Nucleic Acids Res* 2014;42:D191–8.
- [47] Chatr-Aryamontri A, Breitkreutz BJ, Heinicke S, Boucher L, Winter A, Stark C, et al. The BioGRID interaction database: 2013 update. *Nucleic Acids Res* 2013;41:D816–23.
- [48] Jetten AM. Retinoid-related orphan receptors (RORs): critical roles in development, immunity, circadian rhythm, and cellular metabolism. *Nucl Recept Signal* 2009;7:e003.
- [49] Yasumoto H, Meng L, Lin T, Zhu Q, Tsai RY. GNL3L inhibits activity of estrogen-related receptor gamma by competing for coactivator binding. *J Cell Sci* 2007;120:2532–43.
- [50] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013;63:11–30.
- [51] Nyquist MD, Dehm SM. Interplay between genomic alterations and androgen receptor signaling during prostate cancer development and progression. *Horm Cancer* 2013;4:61–9.
- [52] Lonergan PE, Tindall DJ. Androgen receptor signaling in prostate cancer development and progression. *Journal of carcinogenesis*. 2011;10:20.
- [53] Egan A, Dong Y, Zhang H, Qi Y, Balk SP, Sartor O. Castration-resistant prostate cancer: adaptive responses in the androgen axis. *Cancer Treat Rev* 2014;40:426–33.
- [54] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011;61:69–90.
- [55] Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. *Nature* 2000;406:747–52.
- [56] Reis-Filho JS, Westbury C, Pierga JY. The impact of expression profiling on prognostic and predictive testing in breast cancer. *J Clin Pathol* 2006;59:225–31.
- [57] Rouzier R, Wagner P, Morandi P, Pusztai L. Gene expression profiling of primary breast cancer. *Curr Oncol Rep* 2005;7:38–44.
- [58] Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001;98:10869–74.
- [59] Niemeier LA, Dabbs DJ, Beriwal S, Striebel JM, Bhargava R. Androgen receptor in breast cancer: expression in estrogen receptor-positive tumors and in estrogen receptor-negative tumors with apocrine differentiation. *Mod Pathol* 2010;23:205–12.
- [60] Anderson WF, Chatterjee N, Ershler WB, Brawley OW. Estrogen receptor breast cancer phenotypes in the surveillance, epidemiology, and end results database. *Breast Cancer Res Treat* 2002;76:27–36.
- [61] Reis-Filho JS, Tutt AN. Triple negative tumours: a critical review. *Histopathology* 2008;52:108–18.
- [62] Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005;365:1687–717.
- [63] Andre E, Gawlas K, Becker-Andre M. A novel isoform of the orphan nuclear receptor RORbeta is specifically expressed in pineal gland and retina. *Gene* 1998;216:277–83.
- [64] Kojetin DJ, Burris TP. REV-ERB and ROR nuclear receptors as drug targets. *Nat Rev Drug Discovery* 2014;13:197–216.
- [65] Yang XO, Pappu BP, Nurieva R, Akimzhanov A, Kang HS, Chung Y, et al. T helper 17 lineage differentiation is programmed by orphan nuclear receptors RORalpha and RORgamma. *Immunity* 2008;28:29–39.
- [66] Solt LA, Burris TP. Action of RORs and their ligands in (patho)physiology. *Trends Endocrinol Metab* 2012;23:619–27.
- [67] Rahnhotra HS. The interplay between retinoic acid receptor-related orphan receptors and human diseases. *J Recept Signal Transduct Res* 2012;32:181–9.
- [68] Du J, Xu R. RORalpha, a potential tumor suppressor and therapeutic target of breast cancer. *Int J Mol Sci* 2012;13:15755–66.
- [69] Moretti RM, Marelli MM, Motta M, Polizzi D, Monestiroli S, Pratesi G, et al. Activation of the orphan nuclear receptor RORalpha induces growth arrest in androgen-independent DU 145 prostate cancer cells. *Prostate* 2001;46:327–35.
- [70] Moretti RM, Montagnani Marelli M, Motta M, Limonta P. Role of the orphan nuclear receptor ROR alpha in the control of the metastatic behavior of androgen-independent prostate cancer cells. *Oncol Rep* 2002;9:1139–43.
- [71] Wang Y, Kumar N, Nuhant P, Cameron MD, Istrate MA, Roush WR, et al. Identification of SR1078, a synthetic agonist for the orphan nuclear receptors RORalpha and RORgamma. *ACS Chem Biol* 2010;5:1029–34.

- [72] Wang Y, Solt LA, Kojetin DJ, Burris TP. Regulation of p53 stability and apoptosis by a ROR agonist. *PLoS ONE* 2012;7:e34921.
- [73] Zhu Y, McAvoy S, Kuhn R, Smith DI. RORA, a large common fragile site gene, is involved in cellular stress response. *Oncogene* 2006;25:2901–8.
- [74] Lu Y, Yi Y, Liu P, Wen W, James M, Wang D, et al. Common human cancer genes discovered by integrated gene-expression analysis. *PLoS ONE* 2007;2:e1149.
- [75] Xiong G, Wang C, Evers BM, Zhou BP, Xu R. RORalpha suppresses breast tumor invasion by inducing SEMA3F expression. *Cancer Res* 2012;72:1728–39.
- [76] Leris AC, Roberts TR, Jiang WG, Newbold RF, Mokbel K. WNT5A expression in human breast cancer. *Anticancer Res* 2005;25:731–4.
- [77] Kremenevskaja N, von Wasielewski R, Rao AS, Schoff C, Andersson T, Brabant G. Wnt-5a has tumor suppressor activity in thyroid carcinoma. *Oncogene* 2005;24:2144–54.
- [78] Nakshatri H, Bhat-Nakshatri P, Martin DA, Goulet Jr RJ, Sledge Jr GW. Constitutive activation of NF-kappaB during progression of breast cancer to hormone-independent growth. *Mol Cell Biol* 1997;17:3629–39.
- [79] Hayden MS, Ghosh S. Signaling to NF-kappaB. *Genes Dev* 2004;18:2195–224.
- [80] Journiac N, Jolly S, Jarvis C, Gautheron V, Rogard M, Trembleau A, et al. The nuclear receptor ROR(alpha) exerts a bi-directional regulation of IL-6 in resting and reactive astrocytes. *Proc Natl Acad Sci USA* 2009;106:21365–70.
- [81] Delerive P, Monte D, Dubois G, Trottein F, Fruchart-Najib J, Mariani J, et al. The orphan nuclear receptor RORalpha is a negative regulator of the inflammatory response. *EMBO Rep* 2001;2:42–8.
- [82] Kang HS, Angers M, Beak JY, Wu X, Gimble JM, Wada T, et al. Gene expression profiling reveals a regulatory role for RORalpha and RORgamma in phase I and phase II metabolism. *Physiol Genomics* 2007;31:281–94.
- [83] Odawara H, Iwasaki T, Horiguchi J, Rokutanda N, Hirooka K, Miyazaki W, et al. Activation of aromatase expression by retinoic acid receptor-related orphan receptor (ROR)alpha in breast cancer cells: identification of a novel ROR response element. *J Biol Chem* 2009;284:17711–9.
- [84] Kallen JA, Schlaeppi JM, Bitsch F, Geisse S, Geiser M, Delhon I, et al. X-ray structure of the hRORalpha LBD at 1.63 Å: structural and functional data that cholesterol or a cholesterol derivative is the natural ligand of RORalpha. *Structure* 2002;10:1697–707.
- [85] Chuu CP, Kokontis JM, Hiipakka RA, Liao S. Modulation of liver X receptor signaling as novel therapy for prostate cancer. *J Biomed Sci* 2007;14:543–53.
- [86] de Boussac H, Pommier AJ, Dufour J, Trousson A, Caira F, Volle DH, et al. LXR, prostate cancer and cholesterol: the good, the bad and the ugly. *Am J Cancer Res* 2013;3:58–69.
- [87] Fukuchi J, Kokontis JM, Hiipakka RA, Chuu CP, Liao S. Antiproliferative effect of liver X receptor agonists on LNCaP human prostate cancer cells. *Cancer Res* 2004;64:7686–9.
- [88] Fu W, Yao J, Huang Y, Li Q, Li W, Chen Z, et al. LXR agonist regulates the carcinogenesis of PCa via the SOCS3 pathway. *Cell Physiol Biochem* 2014;33:195–204.
- [89] Nguyen-Vu T, Vedin LL, Liu K, Jonsson P, Lin JZ, Candelaria NR, et al. Liver x receptor ligands disrupt breast cancer cell proliferation through an E2F-mediated mechanism. *Breast Cancer Res* 2013;15:R51.
- [90] Vedin LL, Lewandowski SA, Parini P, Gustafsson JA, Steffensen KR. The oxysterol receptor LXR inhibits proliferation of human breast cancer cells. *Carcinogenesis* 2009;30:575–9.
- [91] El Roz A, Bard JM, Huvelin JM, Nazih H. The anti-proliferative and pro-apoptotic effects of the trans9, trans11 conjugated linoleic acid isomer on MCF-7 breast cancer cells are associated with LXR activation. *Prostaglandins Leukot Essent Fatty Acids* 2013;88:265–72.
- [92] El Roz A, Bard JM, Huvelin JM, Nazih H. LXR agonists and ABCG1-dependent cholesterol efflux in MCF-7 breast cancer cells: relation to proliferation and apoptosis. *Anticancer Res* 2012;32:3007–13.
- [93] Hoerer S, Schmid A, Heckel A, Budzinski RM, Nar H. Crystal structure of the human liver X receptor beta ligand-binding domain in complex with a synthetic agonist. *J Mol Biol* 2003;334:853–61.
- [94] Hu B, Bernotas R, Unwalla R, Collini M, Quinet E, Feingold I, et al. Quinoline-3-carboxamide containing sulfones as liver X receptor (LXR) agonists with binding selectivity for LXRbeta and low blood-brain penetration. *Bioorg Med Chem Lett* 2010;20:689–93.
- [95] Hu B, Unwalla RJ, Goljer I, Jetter JW, Quinet EM, Berroddin TJ, et al. Identification of phenylsulfone-substituted quinoxaline (WYE-672) as a tissue selective liver X-receptor (LXR) agonist. *J Med Chem* 2010;53:3296–304.
- [96] Peng D, Hiipakka RA, Dai Q, Guo J, Reardon CA, Getz GS, et al. Antiatherosclerotic effects of a novel synthetic tissue-selective steroidal liver X receptor agonist in low-density lipoprotein receptor-deficient mice. *J Pharmacol Exp Ther* 2008;327:332–42.
- [97] Ratni H, Blum-Kaelin D, Dehmlow H, Hartman P, Jablonski P, Masciadri R, et al. Discovery of tetrahydro-cyclopenta[b]indole as selective LXRs modulator. *Bioorg Med Chem Lett* 2009;19:1654–7.
- [98] Lin FJ, Qin J, Tang K, Tsai SY, Tsai MJ. Coup d'Etat: an orphan takes control. *Endocr Rev* 2011;32:404–21.
- [99] Litchfield LM, Klinge CM. Multiple roles of COUP-TFII in cancer initiation and progression. *J Mol Endocrinol* 2012;49:R135–48.
- [100] Qiu Y, Krishnan V, Zeng Z, Gilbert DJ, Copeland NG, Gibson L, et al. Isolation, characterization, and chromosomal localization of mouse and human COUP-TF I and II genes. *Genomics* 1995;29:204–6.
- [101] Avram D, Ishmael JE, Nevriy DJ, Peterson VJ, Lee SH, Dowell P, et al. Heterodimeric interactions between chicken ovalbumin upstream promoter–transcription factor family members ARP1 and ear2. *J Biol Chem* 1999;274:14331–6.
- [102] Klinge CM, Silver BF, Driscoll MD, Sathya G, Bambara RA, Hilf R. Chicken ovalbumin upstream promoter–transcription factor interacts with estrogen receptor, binds to estrogen response elements and half-sites, and inhibits estrogen-induced gene expression. *J Biol Chem* 1997;272:31465–74.
- [103] Klinge CM. Role of estrogen receptor ligand and estrogen response element sequence on interaction with chicken ovalbumin upstream promoter transcription factor (COUP-TF). *J Steroid Biochem Mol Biol* 1999;71:1–19.
- [104] Metivier R, Gay FA, Hubner MR, Flouriot G, Salbert G, Gannon F, et al. Formation of an hERalpha-COUP-TFI complex enhances hERalpha AF-1 through Ser118 phosphorylation by MAPK. *EMBO J* 2002;21:3443–53.
- [105] Qin J, Wu SP, Creighton CJ, Dai F, Xie X, Cheng CM, et al. COUP-TFII inhibits TGF-beta-induced growth barrier to promote prostate tumorigenesis. *Nature* 2013;493:236–40.
- [106] Song CH, Lee HJ, Park E, Lee K. The chicken ovalbumin upstream promoter–transcription factor II negatively regulates the transactivation of androgen receptor in prostate cancer cells. *PLoS ONE* 2012;7:e49026.
- [107] Perets R, Kaplan T, Stein I, Hidas G, Tayeb S, Avraham E, et al. Genome-wide analysis of androgen receptor targets reveals COUP-TF1 as a novel player in human prostate cancer. *PLoS ONE* 2012;7:e46467.
- [108] Nakshatri H, Mendonca MS, Bhat-Nakshatri P, Patel NM, Goulet Jr RJ, Cornetta K. The orphan receptor COUP-TFII regulates G2/M progression of breast cancer cells by modulating the expression/activity of p21(WAF1/CIP1), cyclin D1, and cdk2. *Biochem Biophys Res Commun* 2000;270:1144–53.
- [109] Riggs KA, Wickramasinghe NS, Cochrum RK, Watts MB, Klinge CM. Decreased chicken ovalbumin upstream promoter transcription factor II expression in tamoxifen-resistant breast cancer cells. *Cancer Res* 2006;66:10188–98.
- [110] Litchfield LM, Appana SN, Datta S, Klinge CM. COUP-TFII inhibits Nf-kappaB activation in endocrine-resistant breast cancer cells. *Mol Cell Endocrinol* 2014;382:358–67.
- [111] Kruse SW, Suino-Powell K, Zhou XE, Kretschman JE, Reynolds R, Vonrhein C, et al. Identification of COUP-TFII orphan nuclear receptor as a retinoic acid-activated receptor. *PLoS Biol* 2008;6:e227.
- [112] Deblois G, Giguere V. Oestrogen-related receptors in breast cancer: control of cellular metabolism and beyond. *Nat Rev Cancer* 2013;13:27–36.
- [113] Bianco S, Lanvin O, Tribollet V, Macari C, North S, Vanacker JM. Modulating estrogen receptor-related receptor-alpha activity inhibits cell proliferation. *J Biol Chem* 2009;284:23286–92.
- [114] Yu S, Wang X, Ng CF, Chen S, Chan FL. ERRgamma suppresses cell proliferation and tumor growth of androgen-sensitive and androgen-insensitive prostate cancer cells and its implication as a therapeutic target for prostate cancer. *Cancer Res* 2007;67:4904–14.
- [115] Yu S, Wong YC, Wang XH, Ling MT, Ng CF, Chen S, et al. Orphan nuclear receptor estrogen-related receptor-beta suppresses in vitro and in vivo growth of prostate cancer cells via p21(WAF1/CIP1) induction and as a potential therapeutic target in prostate cancer. *Oncogene* 2008;27:3313–28.
- [116] Cheung CP, Yu S, Wong KB, Chan LW, Lai FM, Wang X, et al. Expression and functional study of estrogen receptor-related receptors in human prostatic cells and tissues. *J Clin Endocrinol Metab* 2005;90:1830–44.
- [117] Stein RA, Chang CY, Kazmin DA, Way J, Schroeder T, Wergin M, et al. Estrogen-related receptor alpha is critical for the growth of estrogen receptor-negative breast cancer. *Cancer Res* 2008;68:8805–12.
- [118] Kraus RJ, Ariazi EA, Farrell ML, Mertz JE. Estrogen-related receptor alpha 1 actively antagonizes estrogen receptor-regulated transcription in MCF-7 mammary cells. *J Biol Chem* 2002;277:24826–34.
- [119] Heck S, Rom J, Thewes V, Becker N, Blume B, Sinn HP, et al. Estrogen-related receptor alpha expression and function is associated with the transcriptional coregulator AIB1 in breast carcinoma. *Cancer Res* 2009;69:5184–90.
- [120] Surowiak P, Materna V, Gyorffy B, Matkowski R, Wojnar A, Maciejczyk A, et al. Multivariate analysis of estrogen receptor alpha, p52, metallothionein and CD24 expression in invasive breast cancers. *Br J Cancer* 2006;95:339–46.
- [121] Stein RA, Gaillard S, McDonnell DP. Estrogen-related receptor alpha induces the expression of vascular endothelial growth factor in breast cancer cells. *J Steroid Biochem Mol Biol* 2009;114:106–12.
- [122] Ariazi EA, Clark GM, Mertz JE. Estrogen-related receptor alpha and estrogen-related receptor gamma associate with unfavorable and favorable biomarkers, respectively, in human breast cancer. *Cancer Res* 2002;62:6510–8.
- [123] Tiraby C, Hazen BC, Gantner ML, Kralli A. Estrogen-related receptor gamma promotes mesenchymal-to-epithelial transition and suppresses breast tumor growth. *Cancer Res* 2011;71:2518–28.
- [124] Galindo CL, McCormick JF, Bubb VJ, Abid Alkadem DH, Li LS, McIver LJ, et al. A long AAAG repeat allele in the 5' UTR of the ERR-gamma gene is correlated with breast cancer predisposition and drives promoter activity in MCF-7 breast cancer cells. *Breast Cancer Res Treat*. 2011;130:41–8.
- [125] Riggins RB, Lan JP, Zhu Y, Klimach U, Zwart A, Cavalli LR, et al. ERRgamma mediates tamoxifen resistance in novel models of invasive lobular breast cancer. *Cancer Res* 2008;68:8908–17.
- [126] Lu X, Peng L, Lv M, Ding K. Recent advance in the design of small molecular modulators of estrogen-related receptors. *Curr Pharm Des* 2012;18:3421–31.
- [127] Kallen J, Schlaeppi JM, Bitsch F, Filipuzzi I, Schilb A, Riou V, et al. Evidence for ligand-independent transcriptional activation of the human estrogen-related receptor alpha (ERRalpha): crystal structure of ERRalpha ligand binding domain in complex with peroxisome proliferator-activated receptor coactivator-1alpha. *J Biol Chem* 2004;279:49330–7.

- [128] Greschick H, Flaig R, Renaud JP, Moras D. Structural basis for the deactivation of the estrogen-related receptor gamma by diethylstilbestrol or 4-hydroxytamoxifen and determinants of selectivity. *J Biol Chem* 2004;279:33639–46.
- [129] Coward P, Lee D, Hull MV, Lehmann JM. 4-Hydroxytamoxifen binds to and deactivates the estrogen-related receptor gamma. *Proc Natl Acad Sci USA* 2001;98:8880–4.
- [130] Busch BB, Stevens Jr WC, Martin R, Ordentlich P, Zhou S, Sapp DW, et al. Identification of a selective inverse agonist for the orphan nuclear receptor estrogen-related receptor alpha. *J Med Chem* 2004;47:5593–6.
- [131] May FE. Novel drugs that target the estrogen-related receptor alpha: their therapeutic potential in breast cancer. *Cancer Manage Res* 2014;6:225–52.
- [132] Mohan HM, Aherne CM, Rogers AC, Baird AW, Winter DC, Murphy EP. Molecular pathways: the role of NR4A orphan nuclear receptors in cancer. *Clin Cancer Res* 2012;18:3223–8.
- [133] Safe S, Kim K, Li X, Lee S. NR4A orphan receptors and cancer. *Nucl Recept Signal* 2011;9:e002.
- [134] Wilson TE, Fahrner TJ, Johnston M, Milbrandt J. Identification of the DNA binding site for NGFI-B by genetic selection in yeast. *Science (New York, NY)* 1991;252:1296–300.
- [135] Philips A, Lesage S, Gingras R, Maira MH, Gauthier Y, Hugo P, et al. Novel dimeric Nur77 signaling mechanism in endocrine and lymphoid cells. *Mol Cell Biol* 1997;17:5946–51.
- [136] Zhang XK. Targeting Nur77 translocation. *Exp Opin Ther Targets* 2007;11:69–79.
- [137] Li H, Kolluri SK, Gu J, Dawson MI, Cao X, Hobbs PD, et al. Cytochrome c release and apoptosis induced by mitochondrial targeting of nuclear orphan receptor TR3. *Science (New York, NY)* 2000;289:1159–64.
- [138] Wu Q, Liu S, Ye XF, Huang ZW, Su WJ. Dual roles of Nur77 in selective regulation of apoptosis and cell cycle by TPA and ATRA in gastric cancer cells. *Carcinogenesis* 2002;23:1583–92.
- [139] Holmes WF, Soprano DR, Soprano KJ. Early events in the induction of apoptosis in ovarian carcinoma cells by CD437: activation of the p38 MAP kinase signal pathway. *Oncogene* 2003;22:6377–86.
- [140] Moll UM, Marchenko N, Zhang XK. P53 and Nur77/TR3 – transcription factors that directly target mitochondria for cell death induction. *Oncogene* 2006;25:4725–43.
- [141] Ferri KF, Kroemer G. Mitochondria – the suicide organelles. *BioEssays* 2001;23:111–5.
- [142] Zhan Y, Du X, Chen H, Liu J, Zhao B, Huang D, et al. Cytosporone B is an agonist for nuclear orphan receptor Nur77. *Nat Chem Biol* 2008;4:548–56.
- [143] Wang J, Yang J, Zou Y, Huang GL, He ZW. Orphan nuclear receptor nur1 as a potential novel marker for progression in human prostate cancer. *Asian Pac J Cancer Prev* 2013;14:2023–8.
- [144] Cho SD, Lee SO, Chintharlapalli S, Abdelrahim M, Khan S, Yoon K, et al. Activation of nerve growth factor-induced Balpha by methylene-substituted diindolylmethanes in bladder cancer cells induces apoptosis and inhibits tumor growth. *Mol Pharmacol* 2010;77:396–404.
- [145] Chintharlapalli S, Burghardt R, Papineni S, Ramaiah S, Yoon K, Safe S. Activation of Nur77 by selected 1,1-Bis(3'-indolyl)-1-(p-substituted phenyl)methanes induces apoptosis through nuclear pathways. *J Biol Chem* 2005;280:24903–14.
- [146] Wu Q, Dawson MI, Zheng Y, Hobbs PD, Agadir A, Jong L, et al. Inhibition of trans-retinoic acid-resistant human breast cancer cell growth by retinoid X receptor-selective retinoids. *Mol Cell Biol* 1997;17:6598–608.
- [147] Zhan YY, Chen Y, Zhang Q, Zhuang JJ, Tian M, Chen HZ, et al. The orphan nuclear receptor Nur77 regulates LKB1 localization and activates AMPK. *Nat Chem Biol* 2012;8:897–904.
- [148] Wansa KD, Harris JM, Yan C, Ordentlich P, Muscat GE. The AF-1 domain of the orphan nuclear receptor NOR-1 mediates trans-activation, coactivator recruitment, and activation by the purine anti-metabolite 6-mercaptopurine. *J Biol Chem* 2003;278:24776–90.
- [149] Yu RN, Achermann JC, Ito M, Jameson JL. The role of DAX-1 in reproduction. *Trends Endocrinol Metab* 1998;9:169–75.
- [150] Ikeda Y, Swain A, Weber TJ, Hentges KE, Zanaria E, Lalli E, et al. Steroidogenic factor 1 and Dax-1 colocalize in multiple cell lineages: potential links in endocrine development. *Mol Endocrinol* 1996;10:1261–72.
- [151] Gurates B, Amsterdam A, Tamura M, Yang S, Zhou J, Fang Z, et al. WT1 and DAX-1 regulate SF-1-mediated human P450arom gene expression in gonadal cells. *Mol Cell Endocrinol* 2003;208:61–75.
- [152] Iyer AK, McCabe ER. Molecular mechanisms of DAX1 action. *Mol Genet Metab* 2004;83:60–73.
- [153] Nakamura Y, Vargas Morris C, Sasano H, Rainey WE. DAX-1A (NR0B1A) expression levels are extremely low compared to DAX-1 (NR0B1) in human steroidogenic tissues. *Horm Metab Res* 2009;41:30–4.
- [154] Holter E, Kotaja N, Makela S, Strauss L, Kietz S, Janne OA, et al. Inhibition of androgen receptor (AR) function by the reproductive orphan nuclear receptor DAX-1. *Mol Endocrinol* 2002;16:515–28.
- [155] Conde I, Alfaro JM, Fraile B, Ruiz A, Paniagua R, Arenas MI. DAX-1 expression in human breast cancer: comparison with estrogen receptors ER-alpha, ER-beta and androgen receptor status. *Breast Cancer Res* 2004;6:R140–8.
- [156] Zhang H, Thomsen JS, Johansson L, Gustafsson JA, Treuter E. DAX-1 functions as an LXXLL-containing corepressor for activated estrogen receptors. *J Biol Chem* 2000;275:39855–9.
- [157] McCabe ER. DAX1: increasing complexity in the roles of this novel nuclear receptor. *Mol Cell Endocrinol* 2007;265–266:179–82.
- [158] Lalli E, Alonso J. Targeting DAX-1 in embryonic stem cells and cancer. *Exp Opin Ther Targets* 2010;14:169–77.
- [159] Lalli E, Bardoni B, Zazopoulos E, Wurtz JM, Strom TM, Moras D, et al. A transcriptional silencing domain in DAX-1 whose mutation causes adrenal hypoplasia congenita. *Mol Endocrinol* 1997;11:1950–60.
- [160] Sablin EP, Woods A, Krylova IN, Hwang P, Ingraham HA, Fletterick RJ. The structure of corepressor Dax-1 bound to its target nuclear receptor LRH-1. *Proc Natl Acad Sci USA* 2008;105:18390–5.
- [161] Marchler-Bauer A, Zheng C, Chitsaz F, Derbyshire MK, Geer LY, Geer RC, et al. CDD: conserved domains and protein three-dimensional structure. *Nucleic Acids Res* 2013;41:D348–52.
- [162] Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ. Jalview Version 2 – a multiple sequence alignment editor and analysis workbench. *Bioinformatics* 2009;25:1189–91.
- [163] Shaffer PL, Jivan A, Dollins DE, Claessens F, Gewirth DT. Structural basis of androgen receptor binding to selective androgen response elements. *Proc Natl Acad Sci USA* 2004;101:4758–63.
- [164] Chandra V, Huang P, Potluri N, Wu D, Kim Y, Rastinejad F. Multidomain integration in the structure of the HNF-4alpha nuclear receptor complex. *Nature* 2013;495:394–8.
- [165] Pereira de Jesus-Tran K, Cote PL, Cantin L, Blanchet F, Breton R. Comparison of crystal structures of human androgen receptor ligand-binding domain complexed with various agonists reveals molecular determinants responsible for binding affinity. *Protein Sci* 2006;15:987–99.
- [166] Lin KH, Zhu XG, Hsu HC, Chen SL, Shieh HY, Chen ST, et al. Dominant negative activity of mutant thyroid hormone alpha1 receptors from patients with hepatocellular carcinoma. *Endocrinology* 1997;138:5308–15.
- [167] Puzianowska-Kuznicka M, Nauman A, Madej A, Tanski Z, Cheng S, Nauman J. Expression of thyroid hormone receptors is disturbed in human renal clear cell carcinoma. *Cancer Lett* 2000;155:145–52.
- [168] Ando S, Sarlis NJ, Oldfield EH, Yen PM. Somatic mutation of TRbeta can cause a defect in negative regulation of TSH in a TSH-secreting pituitary tumor. *J Clin Endocrinol Metab* 2001;86:5572–6.
- [169] Silva JM, Dominguez G, Gonzalez-Sancho JM, Garcia JM, Silva J, Garcia-Andrade C, et al. Expression of thyroid hormone receptor/erbA genes is altered in human breast cancer. *Oncogene* 2002;21:4307–16.
- [170] Kim WG, Cheng SY. Thyroid hormone receptors and cancer. *Biochim Biophys Acta* 2013;1830:3928–36.
- [171] Soprano DR, Qin P, Soprano KJ. Retinoic acid receptors and cancers. *Annu Rev Nutr* 2004;24:201–21.
- [172] Schneider SM, Offterdinger M, Huber H, Grunt TW. Activation of retinoic acid receptor alpha is sufficient for full induction of retinoid responses in SK-BR-3 and T47D human breast cancer cells. *Cancer Res* 2000;60:5479–87.
- [173] Xu XC, Ro JY, Lee JS, Shin DM, Hong WK, Lotan R. Differential expression of nuclear retinoid receptors in normal, premalignant, and malignant head and neck tissues. *Cancer Res* 1994;54:3580–7.
- [174] Zhang XK, Liu Y, Lee MO, Pfahl M. A specific defect in the retinoic acid response associated with human lung cancer cell lines. *Cancer Res* 1994;54:5663–9.
- [175] McGregor F, Wagner E, Felix D, Soutar D, Parkinson K, Harrison PR. Inappropriate retinoic acid receptor-beta expression in oral dysplasias: correlation with acquisition of the immortal phenotype. *Cancer Res* 1997;57:3886–9.
- [176] Geisen C, Denk C, Gremm B, Baust C, Karger A, Bollag W, et al. High-level expression of the retinoic acid receptor beta gene in normal cells of the uterine cervix is regulated by the retinoic acid receptor alpha and is abnormally down-regulated in cervical carcinoma cells. *Cancer Res* 1997;57:1460–7.
- [177] Wu S, Zhang D, Zhang ZP, Soprano DR, Soprano KJ. Critical role of both retinoid nuclear receptors and retinoid-X-receptors in mediating growth inhibition of ovarian cancer cells by all-trans retinoic acid. *Oncogene* 1998;17:2839–49.
- [178] Qiu H, Zhang W, El-Naggar AK, Lippman SM, Lin P, Lotan R, et al. Loss of retinoic acid receptor-beta expression is an early event during esophageal carcinogenesis. *Am J Pathol* 1999;155:1519–23.
- [179] Lotan Y, Xu XC, Shalev M, Lotan R, Williams R, Wheeler TM, et al. Differential expression of nuclear retinoid receptors in normal and malignant prostates. *J Clin Oncol* 2000;18:1116–21.
- [180] Grommes C, Landreth GE, Sastre M, Beck M, Feinstein DL, Jacobs AH, et al. Inhibition of in vivo glioma growth and invasion by peroxisome proliferator-activated receptor gamma agonist treatment. *Mol Pharmacol* 2006;70:1524–33.
- [181] Muller-Brusselbach S, Ebrahimsade S, Jakes J, Eckhardt J, Rapp UR, Peters JM, et al. Growth of transgenic RAF-induced lung adenomas is increased in mice with a disrupted PPARbeta/delta gene. *Int J Oncol* 2007;31:607–11.
- [182] Matthiessen MW, Pedersen G, Albrektsen T, Adamsen S, Fleckner J, Brynskov J. Peroxisome proliferator-activated receptor expression and activation in normal human colonic epithelial cells and tubular adenomas. *Scand J Gastroenterol* 2005;40:198–205.
- [183] Kim KR, Choi HN, Lee HJ, Baek HA, Park HS, Jang KY, et al. A peroxisome proliferator-activated receptor gamma antagonist induces vimentin cleavage and inhibits invasion in high-grade hepatocellular carcinoma. *Oncol Rep* 2007;18:825–32.
- [184] Dong YW, Wang XP, Wu K. Suppression of pancreatic carcinoma growth by activating peroxisome proliferator-activated receptor gamma involves angiogenesis inhibition. *World J Gastroenterol* 2009;15:441–8.

- [185] Mylona E, Giannopoulou I, Diamantopoulou K, Bakarakos P, Nomikos A, Zervas A, et al. Peroxisome proliferator-activated receptor gamma expression in urothelial carcinomas of the bladder: association with differentiation, proliferation and clinical outcome. *Eur J Surg Oncol* 2009;35:197–201.
- [186] Bonofiglio D, Cione E, Qi H, Pingitore A, Perri M, Catalano S, et al. Combined low doses of PPARgamma and RXR ligands trigger an intrinsic apoptotic pathway in human breast cancer cells. *Am J Pathol* 2009;175:1270–80.
- [187] Hase T, Yoshimura R, Mitsuhashi M, Segawa Y, Kawahito Y, Wada S, et al. Expression of peroxisome proliferator-activated receptors in human testicular cancer and growth inhibition by its agonists. *Urology* 2002;60:542–7.
- [188] Wagner ER, He BC, Chen L, Zuo GW, Zhang W, Shi Q, et al. Therapeutic implications of PPARgamma in human osteosarcoma. *PPAR Res* 2010;956427:16.
- [189] Kurebayashi S, Ueda E, Sakaue M, Patel DD, Medvedev A, Zhang F, et al. Retinoid-related orphan receptor gamma (RORgamma) is essential for lymphoid organogenesis and controls apoptosis during thymopoiesis. *Proc Natl Acad Sci USA* 2000;97:10132–7.
- [190] Swales KE, Korbonits M, Carpenter R, Walsh DT, Warner TD, Bishop-Bailey D. The farnesoid X receptor is expressed in breast cancer and regulates apoptosis and aromatase expression. *Cancer Res* 2006;66:10120–6.
- [191] Maran RR, Thomas A, Roth M, Sheng Z, Esterly N, Pinson D, et al. Farnesoid X receptor deficiency in mice leads to increased intestinal epithelial cell proliferation and tumor development. *J Pharmacol Exp Ther* 2009;328:469–77.
- [192] Lee JY, Lee KT, Lee JK, Lee KH, Jang KT, Heo JS, et al. Farnesoid X receptor, overexpressed in pancreatic cancer with lymph node metastasis promotes cell migration and invasion. *Br J Cancer* 2011;104:1027–37.
- [193] Guan B, Li H, Yang Z, Hoque A, Xu X. Inhibition of farnesoid X receptor controls esophageal cancer cell growth in vitro and in nude mouse xenografts. *Cancer* 2013;119:1321–9.
- [194] Su H, Ma C, Liu J, Li N, Gao M, Huang A, et al. Downregulation of nuclear receptor FXR is associated with multiple malignant clinicopathological characteristics in human hepatocellular carcinoma. *Am J Physiol* 2012;303:G1245–53.
- [195] Ingles SA, Wang J, Coetzee GA, Lee ER, Frankl HD, Haile RW. Vitamin D receptor polymorphisms and risk of colorectal adenomas (United States). *Cancer Causes Control* 2001;12:607–14.
- [196] Andersson P, Varenhorst E, Soderkvist P. Androgen receptor and vitamin D receptor gene polymorphisms and prostate cancer risk. *Eur J Cancer* 2006;42:2833–7.
- [197] McCullough ML, Stevens VL, Diver WR, Feigelson HS, Rodriguez C, Bostick RM, et al. Vitamin D pathway gene polymorphisms, diet, and risk of postmenopausal breast cancer: a nested case–control study. *Breast Cancer Res* 2007;9.
- [198] Zhou J, Liu M, Zhai Y, Xie W. The antiapoptotic role of pregnane X receptor in human colon cancer cells. *Mol Endocrinol* 2008;22:868–80.
- [199] Meyer zu Schwabedissen HE, Tirona RG, Yip CS, Ho RH, Kim RB. Interplay between the nuclear receptor pregnane X receptor and the uptake transporter organic anion transporter polypeptide 1A2 selectively enhances estrogen effects in breast cancer. *Cancer Res* 2008;68:9338–47.
- [200] Gupta D, Venkatesh M, Wang H, Kim S, Sinz M, Goldberg GL, et al. Expanding the roles for pregnane X receptor in cancer: proliferation and drug resistance in ovarian cancer. *Clin Cancer Res* 2008;14:5332–40.
- [201] Huang W, Zhang J, Washington M, Liu J, Parant JM, Lozano G, et al. Xenobiotic stress induces hepatomegaly and liver tumors via the nuclear receptor constitutive androstane receptor. *Mol Endocrinol* 2005;19:1646–53.
- [202] Ning BF, Ding J, Yin C, Zhong W, Wu K, Zeng X, et al. Hepatocyte nuclear factor 4 alpha suppresses the development of hepatocellular carcinoma. *Cancer Res* 2010;70:7640–51.
- [203] Schwartz B, Algamas-Dimantov A, Hertz R, Nataf J, Kerman A, Peri I, et al. Inhibition of colorectal cancer by targeting hepatocyte nuclear factor-4alpha. *Int J Cancer* 2009;124:1081–9.
- [204] Koizume S, Yokota N, Miyagi E, Hirahara F, Nakamura Y, Sakuma Y, et al. Hepatocyte nuclear factor-4-independent synthesis of coagulation factor VII in breast cancer cells and its inhibition by targeting selective histone acetyltransferases. *Mol Cancer Res* 2009;7:1928–36.
- [205] Lee HJ, Hwang M, Chattopadhyay S, Choi HS, Lee K. Hepatocyte nuclear factor-3 alpha (HNF-3alpha) negatively regulates androgen receptor transactivation in prostate cancer cells. *Biochem Biophys Res Commun* 2008;367:481–6.
- [206] Jacobs ET, Martinez ME, Campbell PT, Conti DV, Duggan D, Figueiredo JC, et al. Genetic variation in the retinoid X receptor and calcium-sensing receptor and risk of colorectal cancer in the Colon Cancer Family Registry. *Carcinogenesis* 2010;31:1412–6.
- [207] Wu Q, Li Y, Liu R, Agadir A, Lee MO, Liu Y, et al. Modulation of retinoic acid sensitivity in lung cancer cells through dynamic balance of orphan receptors nur77 and COUP-TF and their heterodimerization. *EMBO J* 1997;16:1656–69.
- [208] Au WW, Abdou-Salama S, Al-Hendy A. Inhibition of growth of cervical cancer cells using a dominant negative estrogen receptor gene. *Gynecol Oncol* 2007;104:276–80.
- [209] Fiorelli G, Picariello L, Martinetti V, Tonelli F, Brandi ML. Functional estrogen receptor beta in colon cancer cells. *Biochem Biophys Res Commun* 1999;261:521–7.
- [210] Wang AG, Lee KY, Kim SY, Choi JY, Lee KH, Kim WH, et al. The expression of estrogen receptors in hepatocellular carcinoma in Korean patients. *Yonsei Med J* 2006;47:811–6.
- [211] Schwartz AG, Pysak GM, Murphy V, Lonardo F, Pass H, Schwartz J, et al. Nuclear estrogen receptor beta in lung cancer: expression and survival differences by sex. *Clin Cancer Res* 2005;11:7280–7.
- [212] Konduri S, Schwarz RE. Estrogen receptor beta/alpha ratio predicts response of pancreatic cancer cells to estrogens and phytoestrogens. *J Surg Res* 2007;140:55–66.
- [213] Walton TJ, Li G, Seth R, McArdle SE, Bishop MC, Rees RC. DNA demethylation and histone deacetylation inhibition co-operate to re-express estrogen receptor beta and induce apoptosis in prostate cancer cell-lines. *Prostate* 2008;68:210–22.
- [214] Cho MA, Lee MK, Nam KH, Chung WY, Park CS, Lee JH, et al. Expression and role of estrogen receptor alpha and beta in medullary thyroid carcinoma: different roles in cancer growth and apoptosis. *J Endocrinol* 2007;195:255–63.
- [215] Nozoe T, Oyama T, Takenoyama M, Hanagiri T, Sugio K, Yasumoto K. Significance of immunohistochemical expression of estrogen receptors alpha and beta in squamous cell carcinoma of the esophagus. *Clin Cancer Res* 2007;13:4046–50.
- [216] Treeck O, Pfeiler G, Mitter D, Latratch C, Piendl G, Ortman O. Estrogen receptor [beta]1 exerts antitumoral effects on SK-OV-3 ovarian cancer cells. *J Endocrinol* 2007;193:421–33.
- [217] Barzon L, Masi G, Pacenti M, Trevisan M, Fallo F, Remo A, et al. Expression of aromatase and estrogen receptors in human adrenocortical tumors. *Virchows Arch* 2008;452:181–91.
- [218] Chen Y, Chen C, Yang B, Xu Q, Wu F, Liu F, et al. Estrogen receptor-related genes as an important panel of predictors for breast cancer response to neoadjuvant chemotherapy. *Cancer Lett* 2011;302:63–8.
- [219] Kassi E, Moutsatsou P. Glucocorticoid receptor signaling and prostate cancer. *Cancer Lett* 2011;302:1–10.
- [220] Vilasco MI, Communal L, Mourra N, Courtin A, Forgez P, Gompel A. Glucocorticoid receptor and breast cancer. *Breast Cancer Res Treat* 2011;130:1–10.
- [221] Kay P, Schlossmacher G, Matthews L, Sommer P, Singh D, White A, et al. Loss of glucocorticoid receptor expression by DNA methylation prevents glucocorticoid induced apoptosis in human small cell lung cancer cells. *PLoS ONE* 2011;6:e24839.
- [222] Békási S, Zalatai A. Overexpression of glucocorticoid receptor in human pancreatic cancer and in xenografts. An immunohistochemical study. *Pathol Oncol Res* 2009;15:561–6.
- [223] Peluso JJ. Progesterone signaling mediated through progesterone receptor membrane component-1 in ovarian cells with special emphasis on ovarian cancer. *Steroids* 2011;76:903–9.
- [224] van der Horst PH, Wang Y, Vandennput I, Kuhne LC, Ewing PC, van Ijcken WF, et al. Progesterone inhibits epithelial-to-mesenchymal transition in endometrial cancer. *PLoS ONE* 2012;7:e30840.
- [225] Gupta P, Agarwal A, Gupta V, Singh PK, Pantola C, Amit S. Expression and clinicopathological significance of estrogen and progesterone receptors in gallbladder cancer. *Gastrointest Cancer Res* 2012;5:41–7.
- [226] Daniel AR, Hagan CR, Lange CA. Progesterone receptor action: defining a role in breast cancer. *Exp Rev Endocrinol Metab* 2011;6:359–69.
- [227] Gonzalez L, Corte M, Vazquez J, Junquera S, Sanchez R, Alvarez A, et al. Androgen receptor expression in breast cancer: relationship with clinicopathological characteristics of the tumors, prognosis, and expression of metalloproteases and their inhibitors. *BMC Cancer* 2008;8:149.
- [228] Dolle L, Adriaenssens E, El Yazidi-Belkoura I, Le Bourhis X, Nurcombe V, Hondermarck H. Nerve growth factor receptors and signaling in breast cancer. *Curr Cancer Drug Targets* 2004;4:463–70.
- [229] Cho SD, Yoon K, Chintharlapalli S, Abdelrahim M, Lei P, Hamilton S, et al. Nur77 agonists induce proapoptotic genes and responses in colon cancer cells through nuclear receptor-dependent and nuclear receptor-independent pathways. *Cancer Res* 2007;67:674–83.
- [230] Fan W, Yanase T, Morinaga H, Gondo S, Okabe T, Nomura M, et al. Atrazine-induced aromatase expression is SF-1 dependent: implications for endocrine disruption in wildlife and reproductive cancers in humans. *Environ Health Perspect* 2007;115:720–7.
- [231] Doghman M, Cazareth J, Douguet D, Madoux F, Hodder P, Lalli E. Inhibition of adrenocortical carcinoma cell proliferation by steroidogenic factor-1 inverse agonists. *J Clin Endocrinol Metab* 2009;94:2178–83.
- [232] Clyne CD, Kovacic A, Speed CJ, Zhou J, Pezzi V, Simpson ER. Regulation of aromatase expression by the nuclear receptor LHR-1 in adipose tissue. *Mol Cell Endocrinol* 2004;215:39–44.
- [233] Schoonjans K, Dubuquoy L, Mebis J, Fayard E, Wendling O, Haby C, et al. Liver receptor homolog 1 contributes to intestinal tumor formation through effects on cell cycle and inflammation. *Proc Natl Acad Sci USA* 2005;102:2058–62.
- [234] Billiard J, Moran RA, Whitley MZ, Chatterjee-Kishore M, Gillis K, Brown EL, et al. Transcriptional profiling of human osteoblast differentiation. *J Cell Biochem* 2003;89:389–400.
- [235] NRNC. A unified nomenclature system for the nuclear receptor superfamily. *Cell* 1999;97:161–3.
- [236] Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SPH, Buneman OP, et al. The IUPHAR/BPS guide to PHARMACOLOGY: an expert-driven knowledgebase of drug targets and their ligands. *Nucleic Acids Res* 2014;42:D1098–106.
- [237] Li L, Liu J, Zhu L, Cutler S, Hasegawa H, Shan B, et al. Discovery and optimization of a novel series of liver X receptor-alpha agonists. *Bioorg Med Chem Lett* 2006;16:1638–42.