Nuclear Receptor Research
Vol. 2 (2015), Article ID 101182, 21 pages
doi:10.11131/2015/101182

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Review Article

# Pan-Cancer Analyses of the Nuclear Receptor Superfamily

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Received 14 August 2015; Accepted 3 November 2015

Editor: Jan-Åke Gustafsson

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**Abstract.** Nuclear receptors (NR) act as an integrated conduit for environmental and hormonal signals to govern genomic responses, which relate to cell fate decisions. We review how their integrated actions with each other, shared co-factors and other transcription factors are disrupted in cancer. Steroid hormone nuclear receptors are oncogenic drivers in breast and prostate cancer and blockade of signaling is a major therapeutic goal. By contrast to blockade of receptors, in other cancers enhanced receptor function is attractive, as illustrated initially with targeting of retinoic acid receptors in leukemia. In the post-genomic era large consortia, such as The Cancer Genome Atlas, have developed a remarkable volume of genomic data with which to examine multiple aspects of nuclear receptor status in a pan-cancer manner. Therefore to extend the review of NR function we have also undertaken bioinformatics analyses of NR expression in over 3000 tumors, spread across six different tumor types (bladder, breast, colon, head and neck, liver and prostate). Specifically, to ask how the NR expression was distorted (altered expression, mutation and CNV) we have applied bootstrapping approaches to simulate data for comparison, and also compared these NR findings to 12 other transcription factor families. Nuclear receptors were uniquely and uniformly downregulated across all six tumor types, more than predicted by chance. These approaches also revealed that each tumor type had a specific NR expression profile but these were most similar between breast and prostate cancer. Some NRs were down-regulated in at least five tumor types (e.g., *NR3C2/MR* and *NR5A2/LRH-1*)) whereas others were uniquely down-regulated in one tumor (e.g., *NR1B3/RARG*). The downregulation was not driven by copy number variation or mutation and epigenetic mechanisms maybe responsible for the altered nuclear receptor expression.

**Keywords**: TCGA; cancer NR3C1/GR NR5A2/LRH-1 NR1B3/RARG; Bootstrap analyses; gene expression; copy number variation; mutation

## 1. Nuclear Receptor Gene Regulatory Actions are Integrated at Multiple Levels

The 48 human NRs form a major network to sense lipophilic molecules from diet, metabolism and hormone production, and regulate genes involved in development, metabolism, circadian rhythm, immune function, proliferation and differentiation [1–6]. Reflecting this central role, they represent the target for approximately 15% of all pharmacologic drugs [7].

The NR superfamily can be classified in several ways. The superfamily can either be sub-divided by phylogenetics [8], or grouped according to the cellular location and ligand

genomic response [9]. In this latter classification, Type I receptors are typically cytoplasmic, associated with heat-shock proteins, and ligand binding induces nuclear translocation. These receptors include the high affinity steroid receptors. Type II receptors, by contrast, are retained in the nucleus in the absence of ligand and continuously participate in chromatin modification events that are reversed upon ligand binding. These receptors are typified by NRs that bind micronutrient ligands, for example, *NR1B1/RARA* and *NR1I1/VDR*. This group can be further sub-divided to include the Type III receptors that bind as homodimers in the absence of ligand at direct repeats and include *NR2A1/HNF4A*. Finally, Type IV receptors bind to DNA through only a single binding domain (as a receptor monomer or dimer); this group includes the orphan receptors *NR1D1/EAR1* and *NR1F1/ROR1*.

Amongst NRs there are well-established examples of co-operative or antagonistic behavior at the gene regulatory level, for instance resulting in the antagonizing transcriptional effects of ERa and RARs in breast cancer cells [10]. Conversely, RXR heterodimerization potentiates the actions of several NRs as illustrated in studies combining 9-*cis* retinoic acid (RXR ligand) with a range of other ligands which has combinatorial effects on cellular phenotypes [11–14] which are mediated through underlying regulation of the global transcriptome [15–18].

The interactions of NRs with coactivators and corepressors has revealed further levels of integration and suggest that gene regulation is dispersed across NRs by virtue of co- factor sharing. Coactivators, such as NCOA3/AIB1, are vital for transactivation by being a platform for the proteins that govern chromatin remodeling and looping, and the sequestration of the basal transcriptional machinery. Similarly, but in an opposite manner, corepressors act to silence or suppress transcription [19–21].

Outside of NR interactions with one another and with corepressors and coactivators, it is also clear that their signaling actions are guided by the actions of pioneer factors such as Forkhead box (FOX) family members [22–24] and integrated with other transcription factor signaling pathways [25], including WNT [26], p53 [27–31], SMADs [32–34] and KLFs [35, 36]. One elegant approach to capture such interactions was undertaken by Novershtern et al. [37] who measured the transcriptome profiles of a large number of hematopoietic stem cells, multiple progenitor states and terminally differentiated cell types. They found distinct regulatory circuits in both stem cells and differentiated cells, and identified 80 distinct modules of tightly co-expressed genes in the hematopoietic system. For example, one module was expressed in granulocytes and monocytes and included genes encoding enzymes and cytokine receptors that are essential for inflammatory responses. Major players in this module were VDR together with the factors CEBPa and SPI1/PU.1. This suggests that the VDR works together with this small set of transcription factors, in order to regulate granulocyte and monocyte differentiation. It is reasonable to anticipate that such modules exist in multiple cell types but are guided by the tissue specific expression of NRs and other factors. As genomic- based approaches are increasingly applied to individual receptors, and groups of NRs, it is becoming ever more clear how their combined actions are central to co-ordinate complex gene regulatory programs that govern cell fate decisions (reviewed in [38]).

## 2. Distorted Nuclear Receptor Function in Cancer

The work of Dr. George Beatson [39] in breast cancer and Dr. Charles Huggins [40] in prostate cancer provided very clear evidence of steroid hormone signaling acting as cancer drivers [41, 42]. Aside from the well-established therapeutic targeting of AR and ERs there are established roles to target GR as a pro-apoptotic therapeutic approach in lymphoma [43–45]. However ligand-activation of the GR has been less effective in other cancers and suggests the biology of the GR is more nuanced with regards to cancer biology. More recently in advanced therapy resistant settings in prostate cancer a role has been revealed for the GR to promote progression by essentially phenocopying the actions of the AR and suggests there are overlapping genomic functions of these receptors [46].

Subsequently, other therapeutic NR roles emerged in cancer and leukemias, not to antagonize but rather to enhance their function. In this case, potentially as pivotal as the discovery of steroid hormone actions as cancer drivers, was the analyses of RAR functions in leukemia that revealed its actions were disrupted, but could be pharmacologically targeted [47].

This was critical for several reasons. Firstly, all-trans retinoic acid (ATRA)-based leukemia therapy represents one of the earliest and most successful examples of targeted therapies and provided a paradigm for other therapies [48–51]; although of course clinical trial success with ATRA preceded cloning of the RARs [52, 53]. Secondly, pioneering work by the groups of Dr. Pier Pelicci [54] and Dr. Ron Evans [55] revealed mechanisms that corrupted RAR signaling in leukemia, specifically in acute promyelocytic leukemia. This leukemia is characterized by translocations of the RARa, including PML-RAR, which generate chimeric receptors that inappropriately retained association with corepressors. This discovery established the premise that altered NR interactions with coactivators and corepressors might have an epigenetic consequence that in turn could be targeted by co-treatment with epigenetic therapies. More widely, the therapeutic success of ATRA in leukemias, in so-called differentiation therapies, was a major catalyst for the explorations of the anticancer actions of other, principally type II, NRs in a wide range of leukemias and solid tumors. In this manner RARs, VDR, PPARs and more recently LXRs and FXR have been considered as potentially druggable

targets across cancers [56–62]. With the increasing number of genomic studies it has also emerged that NRs are disrupted in various tumor types; for example DNA CpG methylation of *RARB* [63–65] and copy number variation of *NR1D1* [66] and *RARA* [67].

More recently, many of these earlier findings have been revisited. For example, reflecting the work of Huggins, the role of ER$\alpha$ and ER$\beta$ signaling in the prostate have been re-investigated and distortions to expression of these receptors appears important [68–70]. Similarly, an appreciation has emerged of the importance of AR signaling in tissues other than the prostate, notably in breast cancer [71, 72].

## 3. Nuclear Receptor Network Approaches in Cancer Cells

Given their interactive nature, various workers have examined NR networks in cancer. For example, profiling approaches using high throughput Q-PCR in breast cancer [73] and *in silico* analyses of prostate cancer data bases [74] both revealed a large complement of NR expressed in tumor and that expression profiles relate to tumor stage. Beyond expression profiling, other investigators have aimed to undertake cistromic analyses of multiple NRs and interacting transcription factors to construct a network level understanding of gene expression programs in breast cancer [10, 75]. These approaches identified high complexity enhancer sites that integrated the actions of multiple NRs and other transcription factors in both direct (*cis*) and indirect (*trans*) and often absent of canonical motifs but associated with significant levels of clustering [76–78].

Cistromic analyses in breast cancer revealed the interactions and cross-talk of multiple NRs and revealed that RAR$\gamma$ was amongst the most commonly found NR binding site with approximately 12000 RAR$\gamma$ binding sites in MCF-7 breast cancer cells. These sites were significantly enriched with other NRs (e.g., RAR$\alpha$, PPAR$\gamma$, VDR, HNF4$\alpha$, ER$\alpha$), pioneer-type factors (FOXA1, SP1, STAT3) and co-regulators (CTCF). Key aspects of these associations, focused around the RAR$\gamma$ and RAR$\alpha$, were related to clinical outcome in breast cancer patients and supported the role of larger networks of NRs to control cell fates. Specifically, the data support the concept of cross-talk between RARs and VDR, which exert mitotic restraint, and other NRs, such as ER$\alpha$, that drive proliferation and survival [10, 75]. Other workers also identified a comparable number of RAR$\gamma$ binding sites, many contained in a so-called Mega-*Trans* complex containing ER$\alpha$ and RAR$\gamma$ at important enhancers in breast cancer [79], and specifically identified a significant role for *trans* RAR$\gamma$ genome binding. The importance of RAR$\gamma$ to regulate ER$\alpha$, has been supported further by RNAi screens in breast cancer cells aimed at dissecting tamoxifen resistance [80].

There is also evidence that NR interactions with coactivators and corepressors are distorted in cancer, which ultimately disrupts NR function. Elevated levels of NCOA3/AIB1 enhance ER$\alpha$ actions in breast cancer through a variety of actions and are associated with worse disease free survival. This has been primarily examined within the context of ER$\alpha$ signaling but is also associated with the actions of other Type 1 receptors including PR, AR, and GR [81–86]. Similarly, the genome-wide binding of the transcriptional co-repressors NCOR1 and NCOR2/SMRT maintains distal enhancer regions in an epigenetically repressed, yet poised, state until released [87, 88]. These corepressors are distorted in many cancers through altered expression levels [89], splice variants [90, 91], mutation status [92] and genetic variation [93], suggesting a prominent role in driving the onco-epigenome. We, and others, have explored the capacity of NCOR1 and NCOR2/SMRT to drive the onco-epigenome by distorting the transcriptional actions for various NRs including several type II receptors such as VDR, PPARs, RARs [3, 89, 94–105].

It is tempting to speculate that there are perhaps more general rules for these interactions, with specificities of coactivators or corepressors for certain types of receptors. However, there are few ChIP-Seq studies for these coactivators and corepressors and largely they have not been analyzed in an unbiased manner. To address this issue we recently undertook an integrative genomics analyses of the NCOR1 cistrome by exploiting ENCODE data [106]. Surprisingly, we found that within the NCOR1 cistrome, NR motifs of any type were not the most commonly enriched, compared to other transcription factors. Of those NR that were enriched, there were both Type 1 (ER$\alpha$) and Type II (PPAR$\gamma$) motifs. This suggests that NCOR1 and NCOR2/SMRT involvement with NR function is either not a major aspect of their function or direct DNA interaction by NR in a cis relationship is limited, and that recruitment may be facilitated by pioneer and other integrating transcription factors. Further integrative and unbiased approaches will be critical in resolving the extent and specificities of coactivator and corepressor interactions in an unbiased manner with NRs and other TFs.

## 4. Integrative Analyses of the Nuclear Receptor Network Expression in Cancer

Given the availability of high quality genomic data for multiple tumors it is possible to investigate NR expression and function individually and in gene networks across different tumor and tissue controls [107–115]. Key to such analyses is the work of The Cancer Genome Atlas (TCGA) [116, 117] which provides genome-wide insight in large numbers of multiple tumor types; at the time of writing this 21441 tumors across 91 cancer studies.

Previously, we exploited the Taylor et al. cohort of prostate cancer tumors [118] available through TCGA and mined the NR network [74]. These analyses revealed that the NR superfamily expression was significantly lost in primary PCa, more than predicted by chance [74]. These findings suggested

a global distortion to the NR superfamily expression in PCa, and given the diversity of NRs detected, that prostate tissue maintenance relies on its ability to sense and respond to a range of hormonal and dietary lipophilic compounds. For instance, members of the RARs, RXRs, LXRs, and PPARs were deregulated in a substantial proportion of these patients.

In parallel, to identify potential mechanisms driving these disruptions we also mined microRNAs that are predicted to target specific NRs, and revealed a significant and reciprocal gain of expression of NR targeting microRNA that was more than predicted by chance. For example, miR-106b was amongst the most upregulated miRNA in our analysis and targets several down regulated NRs, including *PPARA*, suggesting that regulation by miRNAs add yet another layer of complexity within the NR network. Together, these observations of reciprocal NR and miRNA co-expression suggest that epigenetic distortion is important to distort the NR superfamily in the prostate [74, 119–121].

## 5. Pan-Cancer Post-Genomic Assessment of the Nuclear Receptor Superfamily

These findings in the literature, and our own work to date, support the concept that the NR superfamily acts in an integrated manner and is disrupted by multiple mechanisms across cancers. To complement this review we have also collated and analyzed NR expression from multiple TCGA cohorts. We have undertaken a comprehensive analysis of the NR superfamily across multiple cancer types in TCGA with the goal of establishing the tumor-specific and pan-cancer extent to which NRs are distorted. Specifically, from over 3000 tumors spread across six different cancer types we have examined NR expression, copy number variation and mutation status (Figure 1).

*5.1. Pan-cancer analytical methods.* The cancer types selected had matched normal tissue from the tumor-targeted organ and were Bladder (BLCA), Breast (BRCA), Colon (COAD), Head and neck (HNSC), Liver (LIHC), and Prostate (PRAD) cancers. Part of the rationale for choosing these tumor types is that BLCA, COAD, and HNSC are derived from tissues that are all exposed to a wide array of environmental signals, whereas LIHC is derived from a tissue that is the major center of central metabolism and also is exposed to a diverse array of primary metabolites and products of digestion. Finally, PRAD and BRCA are cancers that are driven by steroid hormone signaling.

*Data sets.* All analyses, unless otherwise indicated, were undertaken using the R platform for statistical computing (version 3.1.0) [67], and a range of library packages were implemented in Bioconductor [122]. All transcription factor (TF) family annotations and their inclusive gene identifiers, including NRs were obtained through the HUGO Gene Nomenclature Committee [123]. In the first instance normalized RPKM RNA-seq data from the cohorts were downloaded through the UCSC Cancer Genomics Browser [124] (Figure 1, Table 1, Table 2). Only primary, not metastatic, tumor data was considered, and only NRs that were detectible in at least 80% of normal and tumor samples were included in the expression analyses.

*Statistical Analyses.* To establish if expression levels of NRs and other transcription factors were different in tumors compared to normal samples, we first established the mean and distribution of expression of all genes in pools of normal samples and then calculated the relative expression of genes in tumor samples using Z scores. In this manner, the tumor expression of all genes, including NRs and other TFs, were converted into normal tissue relative Z-scores and significantly altered tumor expression of detectable genes was determined by considering only values that were elevated ($Z \geq 2$) or suppressed ($Z \leq -2$). For copy-number analysis, previously determined copy number variation (CNV) estimates (via the GISTIC 2 method) were directly downloaded and utilized.

For mutation analysis, only transcripts containing protein-coding sequences (CDS) were considered. CDS lengths from all exons associated with a given gene were compiled from Ensembl using BioMart [125]. To correct for mutation frequencies being proportional to the coding length, all regions exons for a given gene were added (including all alternative exons) to yield the total CDS length for each gene. Mutation frequencies (mutations/protein coding base pair) were then calculated utilizing the number of mutations detected across tumors, the number of tumors, and the CDS lengths for all protein coding genes, including NRs and other indicated TF families.

To test if NR superfamily tumor tissue relative expression Z-scores were significantly different than expected by chance we utilized bootstrapping permutation approaches. For instance in BRCA, we determined that the average NR is upregulated ($Z \geq 2$) in 6.72% of tumors, while downregulated ($Z \leq -2$) in 26.05% of tumors (Figure 2, Table 3). Specifically, to test whether these observations were more or less than would be predicted by chance, we applied bootstrap approaches. This approach applies a random sampling method to simulate the distribution of expression changes across the transcriptome for comparison to observed findings [126]. We sampled 100,000 replicates of random gene sets equal to the size of the number of NRs detectable (all NRs detected in >80% tumors and normal samples) in each respective cancer (e.g., 42 in BRCA), within the detectable transcriptome gene set (e.g., $n = 16,622$ in BRCA), and thus determined the distribution of significant relative expression changes across all genes within the patient cohort allowing us to directly compare the observed alterations of NRs.

Similarly, CNV frequency was determined for all genes across the genome (e.g., percentage of tumors with detectable CNV). For instance in BRCA, we observed that the average NR was amplified to some extent in 24.06% of tumors, while

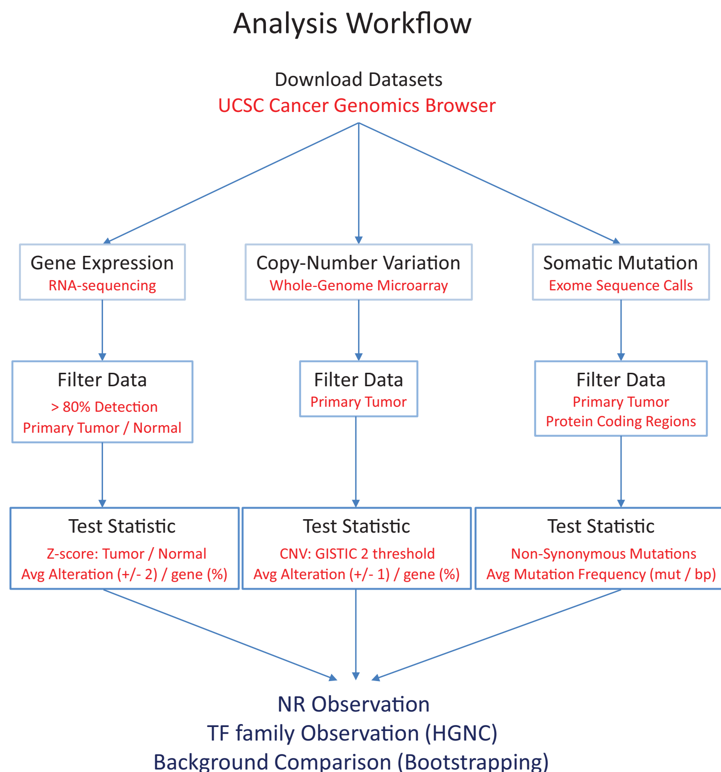


**Figure** 1: Workflow diagram summarizing TCGA analyses. Data were downloaded directly from the UCSC Cancer Genomics Browser (https://genome-cancer.ucsc.edu/), filtered, and global transcriptomic and genomic alterations determined. Observed alterations of NRs and other TF families were directly compared to their genomic background equivalents using a bootstrapping approach.

Table 1: Summary of data sets used for integrative analyses of the NR superfamily across the 6 tumor types indicated; Bladder (BLCA), Breast (BRCA), Colon (COAD), Head and neck (HNSC), Liver (LIHC), and Prostate (PRAD). All normalized data were downloaded directly through the UCSC Cancer Genomics Browser (https://genome-cancer.ucsc.edu/).

| Tumor Type | Dataset ID | Data Type | Tumor (n) | Normal (n) |
|---|---|---|---|---|
| **PRAD** | TCGA_PRAD_exp_HiSeqV2 | Gene Expression | 497 | 52 |
| | TCGA_PRAD_gistic2thd | Copy Number Variation | 492 | NA |
| | TCGA_PRAD_mutation_broad_gene | Somatic Mutation | 425 | NA |
| **BRCA** | TCGA_BRCA_exp_HiSeqV2 | Gene Expression | 1095 | 113 |
| | TCGA_BRCA_gistic2thd | Copy Number Variation | 1079 | NA |
| | TCGA_BRCA_mutation_curated_wustl_gene | Somatic Mutation | 982 | NA |
| **COAD** | TCGA_COAD_exp_HiSeqV2 | Gene Expression | 286 | 41 |
| | TCGA_COAD_gistic2thd | Copy Number Variation | 450 | NA |
| | TCGA_COAD_mutation_bcm_gene | Somatic Mutation | 217 | NA |
| **LIHC** | TCGA_LIHC_exp_HiSeqV2 | Gene Expression | 371 | 42 |
| | TCGA_LIHC_gistic2thd | Copy Number Variation | 370 | NA |
| | TCGA_LIHC_mutation_broad_gene | Somatic Mutation | 202 | NA |
| **HNSC** | TCGA_HNSC_exp_HiSeqV2 | Gene Expression | 519 | 43 |
| | TCGA_HNSC_gistic2thd | Copy Number Variation | 522 | NA |
| | TCGA_HNSC_mutation_broad_gene | Somatic Mutation | 508 | NA |
| **BLCA** | TCGA_BLCA_exp_HiSeqV2 | Gene Expression | 407 | 19 |
| | TCGA_BLCA_gistic2thd | Copy Number Variation | 408 | NA |
| | TCGA_BLCA_mutation_broad_gene | Somatic Mutation | 238 | NA |

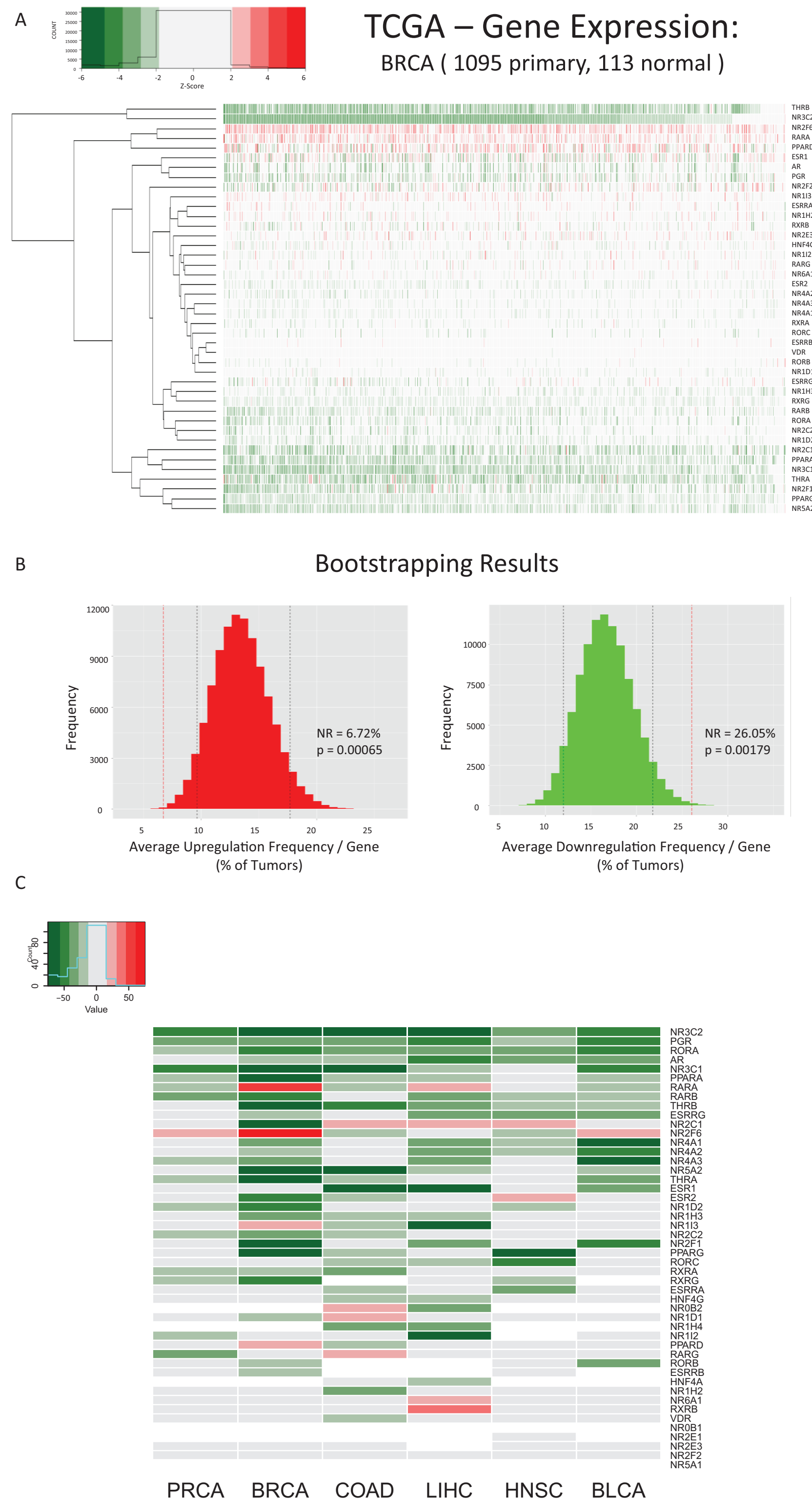


**Figure** 2: Nuclear Receptors are downregulated in cancer. (A) Heatmap depicting the relative expression of all 42 NRs (rows) detectable in 1095 BRCA samples (columns) relative to the expression of NRs observed in a pool of 113 matched normal samples. Tumors with relative expression (Z-score) ≥ +/- 2 are considered significantly upregulated (red) or downregulated (green), respectively. (B) Bootstrapping results comparing the observed mean % of tumors with significantly disrupted expression for NRs relative to the background transcriptome in BRCA. Note that NRs are significantly upregulated less than is predicted by chance ($P = 0.00065$) and significantly downregulated more than is predicted by chance ($P = 0.00179$). (C) Pan-Cancer summary of NR expression patterns. Relative expression scores were determined by summing the Z-scores for a given gene in a given cancer and dividing by the square root of the number of tumors available for that tumor type.

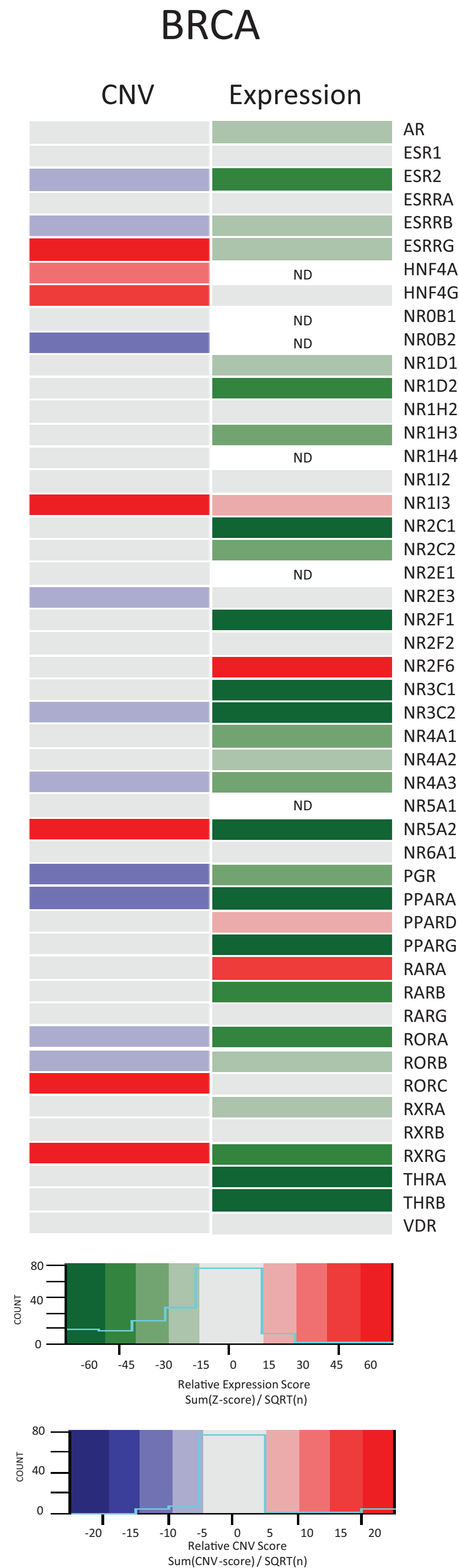


**Figure** 3: Comparing Nuclear Receptor CNV alterations to expression changes in BRCA. Relative scores were determined for CNV alterations (Sum of GISTIC 2 threshold alteration values/square root(number of tumors)) and expression changes (Sum of Z-scores/square root(number of tumors)) for each member of the nuclear receptor superfamily. NRs which were not detectable in >80% of samples are considered not detectable (ND).

Table 2: Summary of transcription factor families, including NRs, utilized in TCGA analyses. Gene families and their members were downloaded from the HUGO Gene Nomenclature Committee (http://www.genenames.org). The average size of TF families examined = 48.15, approximately the size of the NR superfamily.

| **Transcription Factor Family** | **ID** | **n** |
|---|---|---|
| Nuclear hormone receptors | NR | 48 |
| Basic helix-loop-helix proteins | bHLH | 110 |
| Basic leucine zipper proteins | bZIP | 43 |
| E2F transcription factors | E2F | 8 |
| Forkhead boxes | FOX | 43 |
| GATA zinc finger domain containing | GATA | 15 |
| General transcription factors | GeneralTF | 25 |
| Helix-turn-helix ETS type domain containing | ETS | 28 |
| High mobility group | HMG | 15 |
| Homeoboxes | Homeobox | 257 |
| Kruppel-like transcription factors | KLF | 17 |
| SMAD family | SMAD | 8 |
| Sp transcription factors | SP | 9 |

deleted in 20.90% of tumors (Table 5). Likewise, random sampling ($n = 100,000$) was performed to identify the background genomic distribution of copy number alterations in equivalent sized gene sets, to which the NR superfamily observations were directly compared.

Lastly, mutation frequency was determined for all protein coding genes across the genome as described above (mutations/protein coding base pair (bp)). Again, using BRCA as an example, the average NR has a mutation frequency of 1.11E–06 mutations/bp, with the most commonly mutated member being *ESR1* (mutation frequency = 1.72E-06) (Figure 4, Table 4). Random sampling approaches were similarly applied to query this observation against the background protein coding genome.

In all cases, empirical $P$-values were calculated based on the position of determined NR observations relative to the sampling distribution of the genome, to simply determine how probable the observations would be considered likely to occur by random chance. In the same manner stated above, observations and statistical testing were determined for 12 other transcription factor families (Table 2), to serve as comparison. Results of all comparisons are summarized in Table 3 (Expression), Table 4 (CNV), and Table 5 (Mutations).

*5.2. Nuclear receptor superfamily expression is distorted in both common and unique manners across tumors.* The majority of the members of the NR superfamily were expressed across the six tumor types. That is, considering NRs that were expressed at a detectable level (RPKM $>$ 0) in $>$80% of normal and tumor samples revealed that 37 NRs were detected across all six tumor types, and the smallest number expressed was in BLCA which expressed 40 members, whereas the remainder of tissues expressed 42 members. Few tissues shared a common set of NRs, the exception being prostate and breast. Some NRs were undetectable across all tissues, including *NR5A1/SF1* and *NR0B1/DAX1*, while others had distinct patterns of expression including *FXR,* which was detectible only in colon and liver.

Staying with BRCA as an example, Figure 2 shows the relative expression of the 42 NRs across 1095 tumors compared to a pool of 113 matched normal samples. The bootstrap analyses reveal that collectively, NRs are significantly less overexpressed than predicted by chance, and more underexpressed than predicted by chance. This was not a unique event to the BRCA cohort, but rather was seen across all tumor types examined (Table 3). This collective, pan-cancer downregulation was unique to NRs and not observed to a significant extent across all cancer types for any of the other 12 TF families examined. The closest were the KLF and SMAD families. For example, KLF members were collectively and significantly underexpressed in breast, colon and liver cancer types. By contrast, the E2Fs displayed the opposite pattern and were collectively overexpressed in at least three of the tumor types. Other TF families, including the FOX and GATA family members, showed little consensus pattern or were not collectively altered in any cancer. These findings would support the concept that NRs, and interacting TFs that control differentiation such as KLFs and SMADs, are significantly downregulated in a wide spectrum of tumors [32–36].

Next, we sought to compare the patterns of NR expression across tumor types (Figure 2C). To achieve this, a relative expression score was calculated by summing the total Z scores across all tumors of a given cancer type and normalizing each by the square root of the number of tumors available for that respective cancer. Reflecting the fact that as a whole the NR superfamily is significantly downregulated, very few individual NRs had a relative expression score that was positive, and none were positive across all tumors. *NR2F6/EAR2* was over-expressed in BLCA, BRCA, and PRCA which was also reported previously in bladder cancer cell lines [127]. Its expression was also elevated in BRCA and there are several reports of the elevated expression and function of this orphan NR in breast cancer [73] (reviewed in [128]). Also in breast cancer *RARA* and *NR1I3* were elevated. *RARA* is co-amplified with *HER2/ERBB2* [67] and has been proposed to stratify a novel subtype of BRCA [129]. *NR1I3/CAR* was also overexpressed and has not previously been described in breast cancer. *RARA* elevation was also identified in LIHC and there is some evidence to support an oncogenic function for this receptor uniquely in the liver [130]. Another NR to be elevated in 3/6 tumors was the orphan receptor *NR2C1/TR2*, but this appears relatively underexplored in the cancer field (reviewed in [128]). Other examples of NRs showing increased expression in cancers (*NR6A1/GCN1*,

Table 3: Summary of gene expression bootstrapping results comparing the average tumor/normal relative expression (% of tumors displaying overexpression (Z-score $\leq$ 2) and % of tumors displaying underexpression (Z-score $\leq$ -2)) of detectable members of 13 TF families, including NRs, across six different tumor types, relative to the background transcriptome. Shown is the observed value for each TF family (TF AVG), including NRs, as well as the mean value of the transcriptome background (TCGA AVG) for each respective cancer, as well as the bootstrapping results comparing the two. Significantly distorted expression patterns are highlighted in yellow ($P < 0.05$). Note that NRs are less commonly upregulated, and more commonly downregulated, than would be predicted by chance across all six tumor types.

| | | OVEREXPRESSED | | | | UNDEREXPRESSED | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | TF AVG (%) | TCGA AVG (%) | P-value: Less | P-value: More | TF AVG (%) | TCGA AVG (%) | P-value: Less | P-value: More |
| **NR** | **PRAD** | 3.09 | 7.52 | 1.50E-04 | 1.00E+00 | 12.19 | 8.66 | 9.77E-01 | 2.27E-02 |
| | **BRCA** | 6.72 | 13.30 | 6.60E-04 | 9.99E-01 | 26.06 | 16.86 | 9.98E-01 | 1.79E-03 |
| | **COAD** | 4.75 | 18.16 | 1.00E-05 | 1.00E+00 | 30.17 | 19.77 | 9.96E-01 | 3.77E-03 |
| | **LIHC** | 8.87 | 18.34 | 5.00E-05 | 1.00E+00 | 26.92 | 16.61 | 9.99E-01 | 5.90E-04 |
| | **HNSC** | 3.61 | 9.27 | 1.40E-04 | 1.00E+00 | 13.17 | 7.83 | 9.97E-01 | 3.04E-03 |
| | **BLCA** | 2.79 | 10.12 | 1.00E-05 | 1.00E+00 | 25.82 | 11.64 | 1.00E+00 | 2.00E-05 |
| **bHLH** | **PRAD** | 7.12 | 7.52 | 3.68E-01 | 6.31E-01 | 10.93 | 8.66 | 9.62E-01 | 3.76E-02 |
| | **BRCA** | 10.57 | 13.30 | 5.39E-02 | 9.46E-01 | 20.61 | 16.86 | 9.53E-01 | 4.69E-02 |
| | **COAD** | 17.69 | 18.16 | 4.43E-01 | 5.57E-01 | 27.91 | 19.77 | 9.97E-01 | 2.69E-03 |
| | **LIHC** | 16.52 | 18.34 | 1.93E-01 | 8.07E-01 | 18.53 | 16.61 | 8.11E-01 | 1.89E-01 |
| | **HNSC** | 7.78 | 9.27 | 1.47E-01 | 8.53E-01 | 6.42 | 7.83 | 1.30E-01 | 8.70E-01 |
| | **BLCA** | 8.27 | 10.12 | 9.35E-02 | 9.06E-01 | 17.14 | 11.64 | 9.97E-01 | 3.38E-03 |
| **bZIP** | **PRAD** | 4.88 | 7.52 | 2.56E-02 | 9.74E-01 | 10.16 | 8.66 | 8.18E-01 | 1.81E-01 |
| | **BRCA** | 9.01 | 13.30 | 3.47E-02 | 9.65E-01 | 21.93 | 16.86 | 9.36E-01 | 6.38E-02 |
| | **COAD** | 12.15 | 18.16 | 3.72E-02 | 9.63E-01 | 15.93 | 19.77 | 1.42E-01 | 8.58E-01 |
| | **LIHC** | 8.80 | 18.34 | 1.00E-05 | 1.00E+00 | 21.90 | 16.61 | 9.62E-01 | 3.84E-02 |
| | **HNSC** | 5.22 | 9.27 | 7.85E-03 | 9.92E-01 | 6.51 | 7.83 | 2.25E-01 | 7.75E-01 |
| | **BLCA** | 4.52 | 10.12 | 3.70E-04 | 1.00E+00 | 19.99 | 11.64 | 9.98E-01 | 1.64E-03 |
| **E2F** | **PRAD** | 13.30 | 7.52 | 9.43E-01 | 5.72E-02 | 3.60 | 8.66 | 6.55E-02 | 9.34E-01 |
| | **BRCA** | 41.62 | 13.30 | 1.00E+00 | 6.00E-05 | 4.39 | 16.86 | 1.11E-02 | 9.89E-01 |
| | **COAD** | 58.13 | 18.16 | 1.00E+00 | 3.00E-05 | 3.67 | 19.77 | 6.25E-03 | 9.94E-01 |
| | **LIHC** | 51.25 | 18.34 | 1.00E+00 | 3.00E-05 | 1.62 | 16.61 | 1.30E-04 | 1.00E+00 |
| | **HNSC** | 17.99 | 9.27 | 9.62E-01 | 3.84E-02 | 2.75 | 7.83 | 5.25E-02 | 9.47E-01 |
| | **BLCA** | 16.40 | 10.12 | 9.11E-01 | 8.84E-02 | 1.44 | 11.64 | 4.02E-03 | 9.96E-01 |
| **FOX** | **PRAD** | 8.53 | 7.52 | 7.48E-01 | 2.52E-01 | 9.13 | 8.66 | 6.25E-01 | 3.75E-01 |
| | **BRCA** | 17.00 | 13.30 | 9.30E-01 | 7.02E-02 | 19.97 | 16.86 | 7.85E-01 | 2.15E-01 |
| | **COAD** | 20.27 | 18.16 | 7.07E-01 | 2.92E-01 | 21.94 | 19.77 | 7.07E-01 | 2.93E-01 |
| | **LIHC** | 22.55 | 18.34 | 8.93E-01 | 1.07E-01 | 13.49 | 16.61 | 1.91E-01 | 8.08E-01 |
| | **HNSC** | 12.96 | 9.27 | 9.50E-01 | 5.00E-02 | 6.97 | 7.83 | 3.50E-01 | 6.50E-01 |
| | **BLCA** | 10.15 | 10.12 | 5.26E-01 | 4.74E-01 | 11.00 | 11.64 | 4.37E-01 | 5.62E-01 |
| **GATA** | **PRAD** | 6.85 | 7.52 | 4.22E-01 | 5.78E-01 | 6.85 | 8.66 | 2.74E-01 | 7.26E-01 |
| | **BRCA** | 18.96 | 13.30 | 8.77E-01 | 1.23E-01 | 14.67 | 16.86 | 3.86E-01 | 6.14E-01 |
| | **COAD** | 17.10 | 18.16 | 4.66E-01 | 5.34E-01 | 15.06 | 19.77 | 2.57E-01 | 7.42E-01 |
| | **LIHC** | 19.68 | 18.34 | 6.27E-01 | 3.73E-01 | 13.17 | 16.61 | 2.66E-01 | 7.34E-01 |
| | **HNSC** | 7.97 | 9.27 | 3.85E-01 | 6.15E-01 | 3.88 | 7.83 | 7.29E-02 | 9.27E-01 |
| | **BLCA** | 8.45 | 10.12 | 3.38E-01 | 6.62E-01 | 15.57 | 11.64 | 8.18E-01 | 1.82E-01 |
| **GeneralTF** | **PRAD** | 7.70 | 7.52 | 5.65E-01 | 4.35E-01 | 6.80 | 8.66 | 1.96E-01 | 8.04E-01 |
| | **BRCA** | 15.68 | 13.30 | 7.63E-01 | 2.37E-01 | 8.37 | 16.86 | 6.94E-03 | 9.93E-01 |
| | **COAD** | 30.97 | 18.16 | 9.95E-01 | 5.01E-03 | 5.97 | 19.77 | 1.30E-04 | 1.00E+00 |
| | **LIHC** | 23.75 | 18.34 | 9.31E-01 | 6.86E-02 | 10.47 | 16.61 | 3.75E-02 | 9.62E-01 |
| | **HNSC** | 14.10 | 9.27 | 9.66E-01 | 3.39E-02 | 6.81 | 7.83 | 3.46E-01 | 6.54E-01 |
| | **BLCA** | 13.79 | 10.12 | 9.24E-01 | 7.58E-02 | 4.66 | 11.64 | 4.35E-03 | 9.96E-01 |

Table 3: Continued.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **ETS** | **PRAD** | 4.18 | 7.52 | 2.14E-02 | 9.79E-01 | 12.65 | 8.66 | 9.65E-01 | 3.47E-02 |
| | **BRCA** | 6.31 | 13.30 | 3.90E-03 | 9.96E-01 | 17.77 | 16.86 | 6.28E-01 | 3.71E-01 |
| | **COAD** | 18.71 | 18.16 | 5.65E-01 | 4.35E-01 | 23.51 | 19.77 | 7.91E-01 | 2.09E-01 |
| | **LIHC** | 8.96 | 18.34 | 1.49E-03 | 9.99E-01 | 18.21 | 16.61 | 6.77E-01 | 3.23E-01 |
| | **HNSC** | 6.93 | 9.27 | 1.65E-01 | 8.35E-01 | 8.23 | 7.83 | 6.05E-01 | 3.94E-01 |
| | **BLCA** | 5.07 | 10.12 | 9.28E-03 | 9.91E-01 | 8.88 | 11.64 | 1.93E-01 | 8.07E-01 |
| **HMG** | **PRAD** | 9.01 | 7.52 | 7.32E-01 | 2.68E-01 | 10.69 | 8.66 | 7.68E-01 | 2.32E-01 |
| | **BRCA** | 24.11 | 13.30 | 9.86E-01 | 1.42E-02 | 8.34 | 16.86 | 3.70E-02 | 9.63E-01 |
| | **COAD** | 31.29 | 18.16 | 9.76E-01 | 2.35E-02 | 6.19 | 19.77 | 4.71E-03 | 9.95E-01 |
| | **LIHC** | 31.43 | 18.34 | 9.92E-01 | 7.97E-03 | 12.96 | 16.61 | 2.53E-01 | 7.46E-01 |
| | **HNSC** | 19.89 | 9.27 | 9.97E-01 | 3.34E-03 | 8.02 | 7.83 | 5.67E-01 | 4.33E-01 |
| | **BLCA** | 18.53 | 10.12 | 9.88E-01 | 1.24E-02 | 6.32 | 11.64 | 9.42E-02 | 9.06E-01 |
| **Homeobox** | **PRAD** | 7.75 | 7.52 | 6.21E-01 | 3.79E-01 | 7.23 | 8.66 | 5.35E-02 | 9.46E-01 |
| | **BRCA** | 11.78 | 13.30 | 2.00E-01 | 8.00E-01 | 22.57 | 16.86 | 9.99E-01 | 6.20E-04 |
| | **COAD** | 20.88 | 18.16 | 9.10E-01 | 8.99E-02 | 18.99 | 19.77 | 3.56E-01 | 6.43E-01 |
| | **LIHC** | 20.29 | 18.34 | 8.50E-01 | 1.50E-01 | 13.73 | 16.61 | 6.81E-02 | 9.32E-01 |
| | **HNSC** | 17.46 | 9.27 | 1.00E+00 | 1.00E-05 | 4.51 | 7.83 | 1.00E-05 | 1.00E+00 |
| | **BLCA** | 11.07 | 10.12 | 8.05E-01 | 1.95E-01 | 13.83 | 11.64 | 9.32E-01 | 6.75E-02 |
| **KLF** | **PRAD** | 4.01 | 7.52 | 5.79E-02 | 9.42E-01 | 11.00 | 8.66 | 8.07E-01 | 1.93E-01 |
| | **BRCA** | 0.72 | 13.30 | 2.10E-04 | 1.00E+00 | 39.24 | 16.86 | 1.00E+00 | 5.00E-04 |
| | **COAD** | 3.85 | 18.16 | 7.60E-04 | 9.99E-01 | 33.29 | 19.77 | 9.80E-01 | 1.95E-02 |
| | **LIHC** | 3.48 | 18.34 | 2.00E-05 | 1.00E+00 | 29.86 | 16.61 | 9.91E-01 | 8.78E-03 |
| | **HNSC** | 6.65 | 9.27 | 2.01E-01 | 7.98E-01 | 8.66 | 7.83 | 6.53E-01 | 3.47E-01 |
| | **BLCA** | 0.97 | 10.12 | 1.00E-05 | 1.00E+00 | 31.85 | 11.64 | 1.00E+00 | 5.00E-05 |
| **SMAD** | **PRAD** | 1.33 | 7.52 | 5.46E-03 | 9.94E-01 | 24.45 | 8.66 | 9.99E-01 | 6.70E-04 |
| | **BRCA** | 2.09 | 13.30 | 1.49E-03 | 9.99E-01 | 32.33 | 16.86 | 9.77E-01 | 2.26E-02 |
| | **COAD** | 7.78 | 18.16 | 8.74E-02 | 9.12E-01 | 40.08 | 19.77 | 9.86E-01 | 1.39E-02 |
| | **LIHC** | 11.46 | 18.34 | 1.29E-01 | 8.71E-01 | 24.29 | 16.61 | 8.74E-01 | 1.26E-01 |
| | **HNSC** | 7.03 | 9.27 | 3.40E-01 | 6.60E-01 | 10.91 | 7.83 | 8.02E-01 | 1.98E-01 |
| | **BLCA** | 3.22 | 10.12 | 3.07E-02 | 9.69E-01 | 19.47 | 11.64 | 9.02E-01 | 9.76E-02 |
| **SP** | **PRAD** | 4.77 | 7.52 | 2.40E-01 | 7.60E-01 | 10.89 | 8.66 | 7.34E-01 | 2.65E-01 |
| | **BRCA** | 4.69 | 13.30 | 6.00E-02 | 9.40E-01 | 18.84 | 16.86 | 6.39E-01 | 3.60E-01 |
| | **COAD** | 24.59 | 18.16 | 7.68E-01 | 2.32E-01 | 12.00 | 19.77 | 2.25E-01 | 7.75E-01 |
| | **LIHC** | 23.23 | 18.34 | 7.65E-01 | 2.34E-01 | 7.91 | 16.61 | 1.16E-01 | 8.84E-01 |
| | **HNSC** | 3.18 | 9.27 | 7.88E-02 | 9.21E-01 | 6.74 | 7.83 | 4.77E-01 | 5.23E-01 |
| | **BLCA** | 1.60 | 10.12 | 1.04E-02 | 9.90E-01 | 11.71 | 11.64 | 5.62E-01 | 4.38E-01 |

*RARG*, *NR1D1/Rev-Erb-alpha*, *RXRB*) are also relatively unexplored.

Conversely, there were many examples of individual NR expression being lost in cancer. In several instances, NR expression was lost in only some tumor types, or focally in single cancers, suggesting tissue specific importance. One example is *RARG* loss in PRAD, which we identified previously [118] and reflects the prostate metaplasia observed in RAR$\gamma$ null mice [131]. Other examples of specific NR loss that have been previously characterized include *VDR* loss in COAD, *Rev-Erb-alpha* loss in BRCA, and *NR1H2/LXRB* loss in COAD [132–134].

However, other NRs were commonly downregulated in all or most cancers examined, suggesting broader tissue importance. These include examples from all types of NRs, from high affinity classical steroid hormone receptors such as *PR*, *MR*, *GR*; Type II NRs including *RARB*, *PPARA*; to Type III NRs including *NR2F1/COUP-TF1* and *NR2C2/TR2*; and Type IV receptors such as *NR1D2/Rev-erb-beta*. In particular there are a group of eight NRs, which are down-regulated in at least five of the six tumor types (*GR*, *MR*, *PGR*, *AR*, *NR1A2/THRB*, *NR5A2/LRH-1*, *RARB*, *NR1F1/ROR-alpha*).

Some of these are relatively well described and characterized in cancer, such as *RARB* as well as *RORA* as a negative regulator of proliferation and an emerging therapeutic target [135, 136]. However what is perhaps less obvious is why *GR* and *MR* should be so uniformly and strongly reduced in expression. Given the roles for these receptors to contribute to the control of local inflammation, and the importance of inflammation as an early trigger for cancer, it is tempting to

Table 4: Summary of copy number variation bootstrapping results comparing the average copy number alterations (% of tumors with amplification, % of tumors with deletion) observed for 13 TF families, including NRs, across six different tumor types, relative to the background genome. Shown is the observed value for each TF family (TF AVG), including NRs, as well as the mean value of the genome background (TCGA AVG) for each respective cancer, as well as the bootstrapping results comparing the two. Significantly distorted CNV patterns are highlighted in yellow ($P < 0.05$). Note that NRs not commonly amplified or deleted relative to the background genome.

| | | AMPLIFICATIONS | | | | DELETIONS | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | TF AVG (%) | TCGA AVG (%) | P-value: Less | P-value: More | TF AVG (%) | TCGA AVG (%) | P-value: Less | P-value: More |
| NR | PRAD | 5.69 | 5.55 | 5.91E-01 | 4.08E-01 | 6.29 | 8.59 | 1.64E-02 | 9.84E-01 |
| | BRCA | 24.06 | 22.13 | 8.04E-01 | 1.96E-01 | 20.90 | 23.05 | 1.24E-01 | 8.76E-01 |
| | COAD | 16.27 | 18.69 | 1.41E-01 | 8.59E-01 | 15.58 | 16.24 | 3.69E-01 | 6.30E-01 |
| | LIHC | 21.81 | 19.21 | 8.63E-01 | 1.37E-01 | 16.78 | 19.35 | 8.61E-02 | 9.14E-01 |
| | HNSC | 19.80 | 20.65 | 3.47E-01 | 6.53E-01 | 20.85 | 19.79 | 7.05E-01 | 2.95E-01 |
| | BLCA | 25.02 | 24.86 | 5.34E-01 | 4.65E-01 | 24.05 | 24.42 | 4.30E-01 | 5.70E-01 |
| bHLH | PRAD | 6.43 | 5.55 | 9.40E-01 | 5.96E-02 | 6.99 | 8.59 | 1.72E-02 | 9.83E-01 |
| | BRCA | 23.10 | 22.13 | 7.45E-01 | 2.55E-01 | 21.25 | 23.05 | 7.39E-02 | 9.26E-01 |
| | COAD | 20.36 | 18.69 | 8.68E-01 | 1.32E-01 | 16.08 | 16.24 | 4.54E-01 | 5.46E-01 |
| | LIHC | 20.27 | 19.21 | 7.54E-01 | 2.46E-01 | 18.93 | 19.35 | 3.77E-01 | 6.23E-01 |
| | HNSC | 21.31 | 20.65 | 7.02E-01 | 2.98E-01 | 17.43 | 19.79 | 3.66E-02 | 9.63E-01 |
| | BLCA | 26.41 | 24.86 | 8.70E-01 | 1.30E-01 | 23.02 | 24.42 | 1.56E-01 | 8.44E-01 |
| bZIP | PRAD | 5.52 | 5.55 | 5.13E-01 | 4.87E-01 | 6.86 | 8.59 | 8.14E-02 | 9.18E-01 |
| | BRCA | 24.70 | 22.13 | 8.54E-01 | 1.46E-01 | 20.10 | 23.05 | 6.67E-02 | 9.33E-01 |
| | COAD | 19.67 | 18.69 | 6.72E-01 | 3.28E-01 | 13.62 | 16.24 | 8.51E-02 | 9.15E-01 |
| | LIHC | 21.97 | 19.21 | 8.63E-01 | 1.37E-01 | 15.24 | 19.35 | 1.69E-02 | 9.83E-01 |
| | HNSC | 22.48 | 20.65 | 8.11E-01 | 1.89E-01 | 14.88 | 19.79 | 6.99E-03 | 9.93E-01 |
| | BLCA | 26.68 | 24.86 | 7.99E-01 | 2.01E-01 | 21.94 | 24.42 | 1.31E-01 | 8.69E-01 |
| E2F | PRAD | 7.57 | 5.55 | 8.41E-01 | 1.58E-01 | 7.11 | 8.59 | 3.74E-01 | 6.26E-01 |
| | BRCA | 24.08 | 22.13 | 6.73E-01 | 3.27E-01 | 22.37 | 23.05 | 4.63E-01 | 5.37E-01 |
| | COAD | 27.42 | 18.69 | 9.28E-01 | 7.17E-02 | 10.42 | 16.24 | 8.64E-02 | 9.14E-01 |
| | LIHC | 22.64 | 19.21 | 7.43E-01 | 2.56E-01 | 15.44 | 19.35 | 2.09E-01 | 7.91E-01 |
| | HNSC | 25.72 | 20.65 | 8.52E-01 | 1.47E-01 | 13.29 | 19.79 | 8.20E-02 | 9.18E-01 |
| | BLCA | 33.79 | 24.86 | 9.55E-01 | 4.45E-02 | 17.83 | 24.42 | 9.84E-02 | 9.01E-01 |
| FOX | PRAD | 5.03 | 5.55 | 2.92E-01 | 7.07E-01 | 11.22 | 8.59 | 9.68E-01 | 3.23E-02 |
| | BRCA | 20.09 | 22.13 | 2.03E-01 | 7.96E-01 | 24.86 | 23.05 | 8.19E-01 | 1.81E-01 |
| | COAD | 20.69 | 18.69 | 8.04E-01 | 1.96E-01 | 14.57 | 16.24 | 1.97E-01 | 8.03E-01 |
| | LIHC | 18.59 | 19.21 | 4.19E-01 | 5.80E-01 | 19.97 | 19.35 | 6.30E-01 | 3.70E-01 |
| | HNSC | 21.98 | 20.65 | 7.45E-01 | 2.55E-01 | 18.19 | 19.79 | 2.32E-01 | 7.68E-01 |
| | BLCA | 26.13 | 24.86 | 7.25E-01 | 2.74E-01 | 22.32 | 24.42 | 1.73E-01 | 8.27E-01 |
| GATA | PRAD | 7.97 | 5.55 | 9.34E-01 | 6.63E-02 | 8.35 | 8.59 | 5.07E-01 | 4.93E-01 |
| | BRCA | 26.92 | 22.13 | 8.73E-01 | 1.27E-01 | 18.92 | 23.05 | 1.08E-01 | 8.92E-01 |
| | COAD | 22.16 | 18.69 | 8.09E-01 | 1.91E-01 | 17.05 | 16.24 | 6.15E-01 | 3.84E-01 |
| | LIHC | 21.66 | 19.21 | 7.32E-01 | 2.68E-01 | 19.35 | 19.35 | 5.15E-01 | 4.84E-01 |
| | HNSC | 25.87 | 20.65 | 9.19E-01 | 8.08E-02 | 17.28 | 19.79 | 2.56E-01 | 7.44E-01 |
| | BLCA | 31.91 | 24.86 | 9.66E-01 | 3.39E-02 | 18.40 | 24.42 | 5.02E-02 | 9.50E-01 |
| GeneralTF | PRAD | 5.40 | 5.55 | 4.78E-01 | 5.22E-01 | 11.63 | 8.59 | 9.48E-01 | 5.22E-02 |
| | BRCA | 18.32 | 22.13 | 1.09E-01 | 8.91E-01 | 25.30 | 23.05 | 8.10E-01 | 1.90E-01 |
| | COAD | 21.12 | 18.69 | 7.86E-01 | 2.14E-01 | 13.78 | 16.24 | 1.69E-01 | 8.31E-01 |
| | LIHC | 14.57 | 19.21 | 6.68E-02 | 9.33E-01 | 23.68 | 19.35 | 9.42E-01 | 5.84E-02 |
| | HNSC | 20.89 | 20.65 | 5.57E-01 | 4.43E-01 | 19.12 | 19.79 | 4.24E-01 | 5.76E-01 |
| | BLCA | 20.61 | 24.86 | 6.63E-02 | 9.34E-01 | 29.40 | 24.42 | 9.56E-01 | 4.43E-02 |

Table 4: Continued.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ETS | **PRAD** | 5.21 | 5.55 | 4.09E-01 | 5.90E-01 | 8.88 | 8.59 | 6.04E-01 | 3.96E-01 |
| | **BRCA** | 26.81 | 22.13 | 9.32E-01 | 6.82E-02 | 18.58 | 23.05 | 3.01E-02 | 9.70E-01 |
| | **COAD** | 19.11 | 18.69 | 5.77E-01 | 4.23E-01 | 12.71 | 16.24 | 6.58E-02 | 9.34E-01 |
| | **LIHC** | 23.47 | 19.21 | 9.08E-01 | 9.19E-02 | 15.97 | 19.35 | 8.50E-02 | 9.15E-01 |
| | **HNSC** | 19.04 | 20.65 | 2.79E-01 | 7.21E-01 | 20.41 | 19.79 | 6.08E-01 | 3.92E-01 |
| | **BLCA** | 26.60 | 24.86 | 7.43E-01 | 2.56E-01 | 22.07 | 24.42 | 1.95E-01 | 8.05E-01 |
| HMG | **PRAD** | 2.75 | 5.55 | 6.33E-03 | 9.94E-01 | 10.28 | 8.59 | 7.90E-01 | 2.09E-01 |
| | **BRCA** | 15.88 | 22.13 | 4.45E-02 | 9.55E-01 | 25.80 | 23.05 | 7.94E-01 | 2.06E-01 |
| | **COAD** | 14.50 | 18.69 | 1.48E-01 | 8.52E-01 | 19.87 | 16.24 | 8.63E-01 | 1.37E-01 |
| | **LIHC** | 16.67 | 19.21 | 2.95E-01 | 7.05E-01 | 22.68 | 19.35 | 8.33E-01 | 1.67E-01 |
| | **HNSC** | 14.10 | 20.65 | 1.56E-02 | 9.84E-01 | 22.26 | 19.79 | 7.59E-01 | 2.40E-01 |
| | **BLCA** | 17.84 | 24.86 | 2.23E-02 | 9.78E-01 | 27.68 | 24.42 | 8.07E-01 | 1.93E-01 |
| Homebox | **PRAD** | 5.99 | 5.55 | 8.73E-01 | 1.27E-01 | 7.53 | 8.59 | 2.43E-02 | 9.76E-01 |
| | **BRCA** | 21.47 | 22.13 | 2.70E-01 | 7.30E-01 | 21.64 | 23.05 | 5.01E-02 | 9.50E-01 |
| | **COAD** | 20.92 | 18.69 | 9.82E-01 | 1.76E-02 | 14.00 | 16.24 | 2.84E-03 | 9.97E-01 |
| | **LIHC** | 20.18 | 19.21 | 8.14E-01 | 1.86E-01 | 16.51 | 19.35 | 4.60E-04 | 1.00E+00 |
| | **HNSC** | 20.58 | 20.65 | 4.81E-01 | 5.19E-01 | 17.91 | 19.79 | 2.00E-02 | 9.80E-01 |
| | **BLCA** | 25.45 | 24.86 | 7.35E-01 | 2.65E-01 | 22.36 | 24.42 | 1.51E-02 | 9.85E-01 |
| KLF | **PRAD** | 6.68 | 5.55 | 7.99E-01 | 2.01E-01 | 9.07 | 8.59 | 6.35E-01 | 3.64E-01 |
| | **BRCA** | 18.12 | 22.13 | 1.47E-01 | 8.53E-01 | 23.39 | 23.05 | 5.53E-01 | 4.47E-01 |
| | **COAD** | 23.33 | 18.69 | 8.81E-01 | 1.19E-01 | 11.88 | 16.24 | 7.13E-02 | 9.29E-01 |
| | **LIHC** | 15.90 | 19.21 | 2.13E-01 | 7.87E-01 | 20.02 | 19.35 | 5.96E-01 | 4.03E-01 |
| | **HNSC** | 21.22 | 20.65 | 5.98E-01 | 4.02E-01 | 20.81 | 19.79 | 6.37E-01 | 3.63E-01 |
| | **BLCA** | 25.19 | 24.86 | 5.45E-01 | 4.54E-01 | 24.09 | 24.42 | 4.69E-01 | 5.31E-01 |
| SMAD | **PRAD** | 2.21 | 5.55 | 7.35E-03 | 9.93E-01 | 16.34 | 8.59 | 9.80E-01 | 2.01E-02 |
| | **BRCA** | 13.16 | 22.13 | 1.92E-02 | 9.81E-01 | 31.52 | 23.05 | 9.57E-01 | 4.27E-02 |
| | **COAD** | 10.61 | 18.69 | 4.42E-02 | 9.56E-01 | 39.00 | 16.24 | 1.00E+00 | 2.00E-05 |
| | **LIHC** | 10.57 | 19.21 | 3.60E-02 | 9.64E-01 | 26.28 | 19.35 | 9.20E-01 | 7.95E-02 |
| | **HNSC** | 9.89 | 20.65 | 1.18E-03 | 9.99E-01 | 38.36 | 19.79 | 9.99E-01 | 1.14E-03 |
| | **BLCA** | 12.68 | 24.86 | 2.74E-03 | 9.97E-01 | 39.06 | 24.42 | 9.97E-01 | 2.70E-03 |
| SP | **PRAD** | 7.25 | 5.55 | 8.18E-01 | 1.82E-01 | 6.66 | 8.59 | 2.87E-01 | 7.13E-01 |
| | **BRCA** | 20.15 | 22.13 | 3.97E-01 | 6.03E-01 | 18.93 | 23.05 | 1.73E-01 | 8.27E-01 |
| | **COAD** | 27.93 | 18.69 | 9.48E-01 | 5.18E-02 | 6.27 | 16.24 | 1.75E-03 | 9.98E-01 |
| | **LIHC** | 21.17 | 19.21 | 6.72E-01 | 3.28E-01 | 8.89 | 19.35 | 3.09E-03 | 9.97E-01 |
| | **HNSC** | 22.61 | 20.65 | 6.97E-01 | 3.03E-01 | 7.94 | 19.79 | 4.30E-04 | 1.00E+00 |
| | **BLCA** | 27.89 | 24.86 | 7.41E-01 | 2.59E-01 | 15.44 | 24.42 | 2.83E-02 | 9.72E-01 |

speculate that the common loss of these receptors reflects this central role. This surprising finding was also identified in breast cancer by high throughput Q-PCR approaches which revealed that *MR*, along with *THRB* and *PPARG* were altered and significantly predicted metastasis-free survival in patients [73].

*5.3. Neither copy number variation nor mutation fully explain the changes in NR expression.* Subsequently, we examined how genomic alterations including CNV and mutations associated with the expression changes observed within the NR superfamily. Collectively, CNV changes in the NR superfamily were not significantly different from the genome background in each of the six tumor types considered (Table 4). This was generally true of the other TF families considered with the exception of the SMADs, which were significantly less amplified and more deleted than expected by chance across all the tumor types. This suggests a common mechanism driving downregulation of some transcription factors including SMADs may be result of genomic instability, while others including NRs may be results of additional mechanisms.

However, comparing the results of CNV alterations with gene expression changes within an individual tumor reveals some NR specific associations for individual receptors, as can be seen comparing relative expression scores to relative CNV scores (calculated in a similar fashion to relative expression scores, as described above) in BRCA (Figure 3). From this comparison it appears that whilst there is

Table 5: Summary of somatic mutation bootstrapping results comparing the protein coding sequence mutation frequencies (mutations/coding base pair) of members of 13 TF families, including NRs, across six different tumor types relative to the protein coding genome. Shown is the observed mutation frequency for each TF family (TF AVG), including NRs, as well as the mean value of the protein coding genome background (TCGA AVG) for each respective cancer, as well as the bootstrapping results comparing the two. Significantly distorted mutation frequencies are highlighted in yellow ($P < 0.05$). Note that coding sequences of NRs not commonly mutated relative to the background protein coding genome. Also, the most commonly mutated member of each TF family is listed for each respective cancer, along with its respective mutation frequency and how it relates to the background mutation frequency (mutation ratio).

| | | Somatic Mutations | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Avg. Frequency (mut/bp) | | Bootstrapping P-values: | | Top Mutated Gene | | | |
| | | TF fam | TCGA | Less | More | Gene | % of Tumors | Mutation Frequency | Mutation Ratio: relative to background |
| NR | PRAD | 7.31E-07 | 8.76E-07 | 3.42E-01 | 6.58E-01 | RXRB | 0.71 | 5.12E-07 | 0.58 |
| | BRCA | 1.11E-06 | 1.32E-06 | 1.61E-01 | 8.39E-01 | ESR1 | 0.72 | 1.72E-06 | 1.31 |
| | COAD | 6.59E-06 | 6.33E-06 | 6.43E-01 | 3.57E-01 | AR | 12.90 | 1.92E-05 | 3.03 |
| | LIHC | 1.17E-05 | 1.51E-05 | 2.80E-02 | 9.72E-01 | RXRA | 4.95 | 3.56E-05 | 2.35 |
| | HNSC | 2.92E-06 | 3.11E-06 | 4.10E-01 | 5.90E-01 | RARG | 2.17 | 7.79E-06 | 2.50 |
| | BLCA | 5.34E-06 | 4.82E-06 | 8.01E-01 | 1.99E-01 | RXRA | 6.72 | 4.84E-05 | 10.05 |
| bHLH | PRAD | 8.10E-07 | 8.75E-07 | 4.49E-01 | 5.51E-01 | MESP2 | 0.94 | 6.30E-06 | 7.20 |
| | BRCA | 1.34E-06 | 1.32E-06 | 6.39E-01 | 3.61E-01 | NPAS4 | 0.92 | 3.56E-06 | 2.70 |
| | COAD | 7.66E-06 | 6.33E-06 | 9.67E-01 | 3.27E-02 | TCF15 | 5.53 | 4.62E-05 | 7.30 |
| | LIHC | 1.45E-05 | 1.51E-05 | 3.37E-01 | 6.63E-01 | NCOA3 | 5.45 | 1.14E-05 | 0.75 |
| | HNSC | 3.17E-06 | 3.11E-06 | 6.37E-01 | 3.63E-01 | SIM1 | 3.94 | 1.23E-05 | 3.94 |
| | BLCA | 6.20E-06 | 4.81E-06 | 9.79E-01 | 2.11E-02 | NCOA1 | 5.88 | 1.27E-05 | 2.65 |
| bZIP | PRAD | 7.14E-07 | 8.74E-07 | 3.24E-01 | 6.76E-01 | BACH2 | 0.47 | 1.45E-06 | 1.66 |
| | BRCA | 1.26E-06 | 1.32E-06 | 4.58E-01 | 5.42E-01 | BACH2 | 1.23 | 3.78E-06 | 2.87 |
| | COAD | 3.45E-06 | 6.33E-06 | 3.60E-04 | 1.00E+00 | BACH2 | 2.76 | 8.50E-06 | 1.34 |
| | LIHC | 1.13E-05 | 1.51E-05 | 1.91E-02 | 9.81E-01 | NFE2L2 | 4.95 | 8.89E-06 | 0.59 |
| | HNSC | 2.49E-06 | 3.11E-06 | 1.24E-01 | 8.76E-01 | NFE2L2 | 5.12 | 9.19E-06 | 2.96 |
| | BLCA | 4.16E-06 | 4.80E-06 | 2.33E-01 | 7.67E-01 | NFE2L2 | 6.30 | 1.13E-05 | 2.36 |
| E2F | PRAD | 2.31E-07 | 8.80E-07 | 6.95E-02 | 9.30E-01 | E2F3 | 0.24 | 1.09E-06 | 1.24 |
| | BRCA | 1.28E-06 | 1.32E-06 | 5.45E-01 | 4.55E-01 | E2F1 | 0.51 | 3.89E-06 | 2.96 |
| | COAD | 5.16E-06 | 6.35E-06 | 3.38E-01 | 6.62E-01 | E2F7 | 3.23 | 8.34E-06 | 1.31 |
| | LIHC | 1.49E-05 | 1.51E-05 | 5.57E-01 | 4.43E-01 | E2F8 | 3.96 | 1.28E-05 | 0.85 |
| | HNSC | 2.49E-06 | 3.11E-06 | 3.47E-01 | 6.53E-01 | E2F5 | 1.18 | 5.10E-06 | 1.64 |
| | BLCA | 4.77E-06 | 4.81E-06 | 5.66E-01 | 4.34E-01 | E2F8 | 3.78 | 1.22E-05 | 2.54 |
| FOX | PRAD | 2.07E-06 | 8.74E-07 | 9.90E-01 | 1.05E-02 | FOXA1 | 5.88 | 3.84E-05 | 44.00 |
| | BRCA | 1.12E-06 | 1.32E-06 | 1.92E-01 | 8.08E-01 | FOXA1 | 2.35 | 1.54E-05 | 11.69 |
| | COAD | 6.33E-06 | 6.33E-06 | 5.46E-01 | 4.54E-01 | FOXO4 | 3.23 | 1.78E-05 | 2.81 |
| | LIHC | 1.45E-05 | 1.51E-05 | 4.30E-01 | 5.70E-01 | FOXP2 | 4.95 | 1.06E-05 | 0.70 |
| | HNSC | 2.96E-06 | 3.11E-06 | 4.51E-01 | 5.49E-01 | FOXJ2 | 1.77 | 8.95E-06 | 2.88 |
| | BLCA | 5.59E-06 | 4.81E-06 | 8.61E-01 | 1.39E-01 | FOXA1 | 4.62 | 3.02E-05 | 6.28 |
| GATA | PRAD | 4.52E-07 | 8.73E-07 | 1.37E-01 | 8.63E-01 | TRPS1 | 0.71 | 6.16E-07 | 0.71 |
| | BRCA | 4.44E-06 | 1.32E-06 | 9.96E-01 | 4.16E-03 | GATA3 | 9.93 | 5.63E-05 | 42.74 |
| | COAD | 7.49E-06 | 6.32E-06 | 7.86E-01 | 2.14E-01 | TRPS1 | 9.68 | 8.45E-06 | 1.34 |
| | LIHC | 1.09E-05 | 1.52E-05 | 8.94E-02 | 9.11E-01 | TRPS1 | 6.44 | 5.62E-06 | 0.37 |
| | HNSC | 2.31E-06 | 3.11E-06 | 1.85E-01 | 8.15E-01 | TRPS1 | 2.36 | 2.06E-06 | 0.66 |
| | BLCA | 4.03E-06 | 4.81E-06 | 3.11E-01 | 6.89E-01 | TRPS1 | 5.88 | 5.14E-06 | 1.07 |
| GeneralTF | PRAD | 3.87E-07 | 8.73E-07 | 3.43E-02 | 9.66E-01 | ERCC2 | 0.71 | 2.30E-06 | 2.64 |
| | BRCA | 1.07E-06 | 1.32E-06 | 2.00E-01 | 8.00E-01 | GTF3C1 | 1.23 | 1.47E-06 | 1.12 |
| | COAD | 7.01E-06 | 6.33E-06 | 7.35E-01 | 2.65E-01 | TBP | 17.97 | 7.11E-05 | 11.23 |

Table 5: Continued.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **LIHC** | 1.57E-05 | 1.51E-05 | 6.26E-01 | 3.74E-01 | GTF3C1 | 5.45 | 6.52E-06 | 0.43 |
| | **HNSC** | 3.30E-06 | 3.12E-06 | 6.74E-01 | 3.26E-01 | GTF3C1 | 2.95 | 3.53E-06 | 1.13 |
| | **BLCA** | 5.32E-06 | 4.80E-06 | 7.48E-01 | 2.52E-01 | ERCC2 | 9.24 | 3.01E-05 | 6.27 |
| **ETS** | **PRAD** | 9.02E-07 | 8.76E-07 | 6.44E-01 | 3.56E-01 | ERF | 0.71 | 2.23E-06 | 2.54 |
| | **BRCA** | 1.72E-06 | 1.32E-06 | 9.35E-01 | 6.54E-02 | ETV5 | 0.92 | 3.99E-06 | 3.03 |
| | **COAD** | 7.82E-06 | 6.33E-06 | 8.86E-01 | 1.14E-01 | ETV3L | 2.76 | 2.55E-05 | 4.02 |
| | **LIHC** | 1.48E-05 | 1.51E-05 | 5.08E-01 | 4.92E-01 | ETV3L | 3.96 | 3.65E-05 | 2.41 |
| | **HNSC** | 3.39E-06 | 3.11E-06 | 7.22E-01 | 2.78E-01 | SPDEF | 1.38 | 1.37E-05 | 4.39 |
| | **BLCA** | 6.81E-06 | 4.81E-06 | 9.66E-01 | 3.42E-02 | ELF3 | 11.76 | 7.51E-05 | 15.61 |
| **HMG** | **PRAD** | 5.64E-07 | 8.76E-07 | 2.51E-01 | 7.49E-01 | HMGB2 | 0.47 | 4.50E-06 | 5.14 |
| | **BRCA** | 1.80E-06 | 1.32E-06 | 9.22E-01 | 7.79E-02 | HMGXB4 | 0.72 | 2.03E-06 | 1.54 |
| | **COAD** | 4.99E-06 | 6.33E-06 | 2.23E-01 | 7.77E-01 | HMG20A | 2.30 | 1.35E-05 | 2.13 |
| | **LIHC** | 1.84E-05 | 1.51E-05 | 8.41E-01 | 1.59E-01 | HMGXB3 | 4.46 | 7.91E-06 | 0.52 |
| | **HNSC** | 2.86E-06 | 3.11E-06 | 4.54E-01 | 5.46E-01 | HMGXB4 | 1.57 | 4.46E-06 | 1.43 |
| | **BLCA** | 5.59E-06 | 4.80E-06 | 7.79E-01 | 2.21E-01 | HMGB2 | 2.10 | 2.01E-05 | 4.18 |
| **Homeobox** | **PRAD** | 1.14E-06 | 8.74E-07 | 9.51E-01 | 4.95E-02 | ZFHX3 | 3.06 | 2.64E-06 | 3.02 |
| | **BRCA** | 1.35E-06 | 1.32E-06 | 6.80E-01 | 3.20E-01 | ZFHX4 | 3.48 | 2.05E-06 | 1.56 |
| | **COAD** | 7.87E-06 | 6.33E-06 | 9.88E-01 | 1.23E-02 | ZFHX4 | 17.51 | 1.03E-05 | 1.63 |
| | **LIHC** | 1.60E-05 | 1.51E-05 | 8.13E-01 | 1.87E-01 | ZFHX4 | 8.42 | 4.96E-06 | 0.33 |
| | **HNSC** | 3.51E-06 | 3.11E-06 | 9.35E-01 | 6.50E-02 | ZFHX4 | 8.46 | 4.99E-06 | 1.60 |
| | **BLCA** | 5.54E-06 | 4.81E-06 | 9.55E-01 | 4.52E-02 | ZFHX4 | 12.61 | 7.43E-06 | 1.55 |
| **KLF** | **PRAD** | 4.01E-07 | 8.76E-07 | 8.01E-02 | 9.20E-01 | KLF4 | 0.47 | 1.78E-06 | 2.03 |
| | **BRCA** | 1.07E-06 | 1.32E-06 | 2.50E-01 | 7.50E-01 | KLF15 | 0.72 | 5.73E-06 | 4.35 |
| | **COAD** | 8.59E-06 | 6.33E-06 | 9.16E-01 | 8.41E-02 | KLF1 | 5.07 | 4.65E-05 | 7.36 |
| | **LIHC** | 1.61E-05 | 1.51E-05 | 6.77E-01 | 3.23E-01 | KLF15 | 5.94 | 4.75E-05 | 3.14 |
| | **HNSC** | 2.64E-06 | 3.11E-06 | 3.25E-01 | 6.75E-01 | KLF3 | 0.98 | 8.25E-06 | 2.65 |
| | **BLCA** | 4.23E-06 | 4.80E-06 | 3.68E-01 | 6.32E-01 | KLF5 | 6.30 | 2.61E-05 | 5.44 |
| **SMAD** | **PRAD** | 1.05E-06 | 8.79E-07 | 7.32E-01 | 2.68E-01 | SMAD4 | 0.94 | 2.75E-06 | 3.13 |
| | **BRCA** | 1.29E-06 | 1.31E-06 | 5.56E-01 | 4.44E-01 | SMAD2 | 0.72 | 2.57E-06 | 1.95 |
| | **COAD** | 1.02E-05 | 6.31E-06 | 9.43E-01 | 5.67E-02 | SMAD4 | 13.36 | 3.90E-05 | 6.18 |
| | **LIHC** | 9.19E-06 | 1.51E-05 | 6.62E-02 | 9.34E-01 | SMAD5 | 3.47 | 1.10E-05 | 0.72 |
| | **HNSC** | 2.58E-06 | 3.12E-06 | 3.85E-01 | 6.15E-01 | SMAD4 | 2.56 | 7.47E-06 | 2.40 |
| | **BLCA** | 4.40E-06 | 4.81E-06 | 4.73E-01 | 5.27E-01 | SMAD3 | 2.10 | 7.31E-06 | 1.52 |
| **SP** | **PRAD** | 1.13E-06 | 8.77E-07 | 7.82E-01 | 2.18E-01 | SP8 | 1.18 | 2.69E-06 | 3.06 |
| | **BRCA** | 8.52E-07 | 1.32E-06 | 1.30E-01 | 8.70E-01 | SP3 | 0.72 | 2.62E-06 | 1.98 |
| | **COAD** | 6.68E-06 | 6.33E-06 | 6.24E-01 | 3.76E-01 | SP8 | 4.61 | 1.05E-05 | 1.66 |
| | **LIHC** | 1.15E-05 | 1.52E-05 | 2.13E-01 | 7.87E-01 | SP3 | 4.95 | 1.81E-05 | 1.19 |
| | **HNSC** | 1.85E-06 | 3.12E-06 | 1.06E-01 | 8.94E-01 | SP4 | 1.18 | 4.62E-06 | 1.48 |
| | **BLCA** | 2.94E-06 | 4.80E-06 | 1.16E-01 | 8.84E-01 | SP1 | 2.10 | 5.48E-06 | 1.14 |

a reasonable agreement between copy number loss and reduced or absent gene expression, the reverse is not true. For instance, in the BRCA cohort, nine out of ten NRs that are significantly reduced at the genomic level for the receptor are also reduced or absent in expression, including *NR3A2/ESR2* and *PGR*. By contrast of the seven NRs that are amplified, only one results in elevated gene expression, namely *NR1I3*. Meanwhile for the other six NRs associated with amplified regions there is no significant gain, and in fact there is still significant loss of expression in several cases, suggesting other regulatory events control expression from the amplicon.

Finally, examination of the mutational status of the NR superfamily did not reveal a significant relationship when considering the number of mutations, after normalizing for the length of the total coding sequence, as compared to the background genome (Figure 4, Table 5). There were not many consistent patterns across cancers concerning TF family mutation frequencies, with the exception of Homeobox TFs which are more commonly mutated than predicted by chance in 3 cancers. Of the focal examples of commonly mutated families in specific cancers such as FOXs in PRAD and GATAs in BRCA, they are largely driven by one or two commonly mutated members, of which there is some

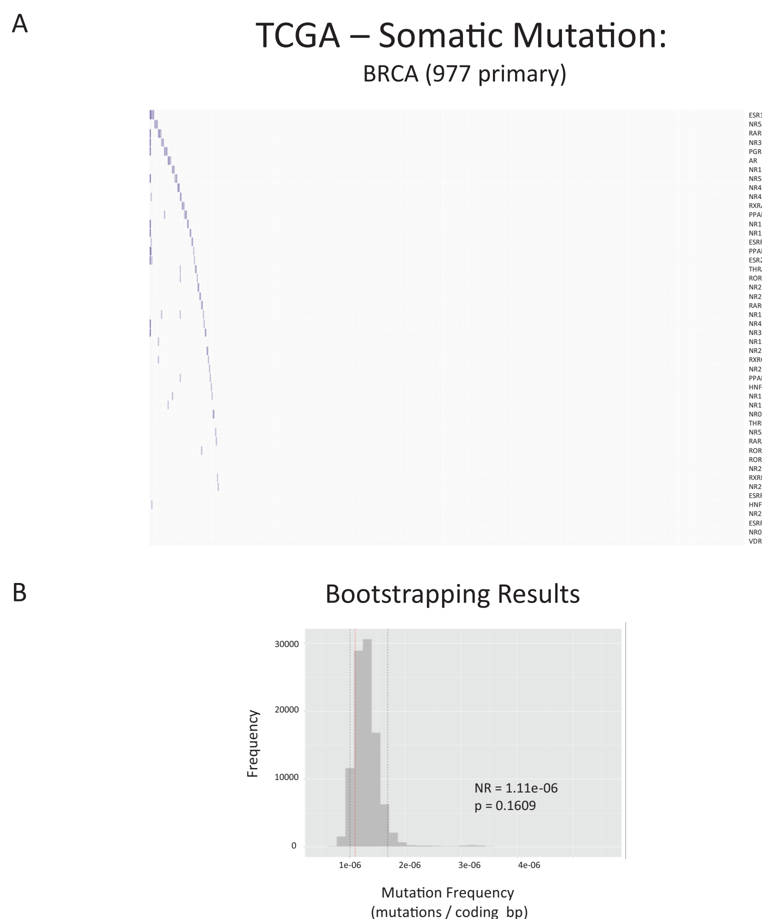


**Figure** 4: Nuclear Receptors are not common targets of somatic mutation in cancer. (A) Heatmap depicting non-synonymous mutations found in protein coding regions for all 48 NRs (rows) in 977 primary BRCA samples (columns). Observed mutations are depicted in blue. (B) Bootstrapping results comparing the observed mutation frequency (mutations/protein coding base pair) for NRs relative to the background protein coding genome in BRCA. Note that NRs are not significantly mutated more or less than is predicted by chance.

precedent in the literature; *FOXA1* is commonly mutated in prostate cancer [137], *GATA3* is commonly mutated in breast cancer [138], although for others such as *ELF3* (ETS family) in BLCA there doesn't appear to be any literature to date.

## 6. Conclusions and Future Perspectives

The present review has described how the NR superfamily is integrated through shared genomic binding, shared pathways of genomic signaling, shared cofactor interactions and cross-talk with other TF pathways. Signaling by NRs is central to many cell fate decisions and as a consequence these actions are corrupted in many cancer cell types. Indeed the history of cancer research is intimately interwoven with elucidation of NR function from the earliest studies of steroidal signaling in breast and prostate cancer, to the identification of targeted therapies, to discovery of epigenetic mechanisms that distort gene regulation function and now, in the post-genomic era, to development of integrative genomic workflows that combine genomic, epigenomic and transcriptomic data to develop dynamic maps of NR signaling.

We sought to build on the review of NR function by developing a pan-cancer view of NR expression by exploiting the remarkable volume of genomic data developed by TCGA. Transcriptomic and genomic alterations of the NR superfamily across six tumor types were examined and by exploiting bootstrapping approaches we were able to generate robust statistical statements concerning the expression, CNV and mutation status of the NR superfamily, alongside 12 other TF families as comparison.

A clear finding from these approaches was that the detected members of the NR superfamily are more down-regulated than predicted by chance, an observation which was uniform across the cancers examined. No other TF family displayed this phenomenon, although KLFs and SMADs mirrored it to a more restricted and limited statistical extent and reflects studies that identified cross-talk between NRs/SMADs/KLFs. Within each tumor however the precise up and down regulated NRs varied, and comparing across

cancers revealed the common downregulation of *GR*, *MR*, *PGR,* and *THRB*, whereas other changes in expression appeared be unique to a specific tumor type; *RARG* loss in prostate and gain in colon cancer, gain of *NR6A1* and *RXRB* in liver cancer, loss of *VDR* in colon cancer; the gain in colon and loss in breast cancer of *Rev-erb-alpha* and the loss in colon of *LXRB*.

Interestingly, whilst NRs were strongly downregulated, this was only to a small extent, if at all, explained by genomic causes such as CNV or mutation, as opposed to other TF families including SMADs whose expression changes reflected CNV alterations. Therefore, an interesting implication of this observation is the idea that epigenomic, rather than genomic, mechanisms may be the drivers for this phenomenon, and possibly that while NRs are down-regulated in cancer, they may remain functional. There are well-established roles for DNA methylation to down-regulate NRs, most notably *RARB* [63–65], and the current findings suggest that targeted DNA methylation may be responsible for suppression of other NRs. Previously, we have considered roles for microRNA to explain NR expression levels and established that certain cohorts of NR targeting miRNA were more up-regulated than predicted by chance [74]. Whilst undertaking these studies is statistically more challenging, given the many to many relationships between miRNA and mRNA, it seems reasonable to suggest that networks of miRNA may play a significant role to distort NR network expression and therefore function across cancers.

Interestingly, all tissues examined expressed a broad array of NRs, with BLCA expressing the fewest at 40. This observation coincides with recent undertakings profiling NRs in tissues. For instance, the 42 NRs detected in BRCA correlate well with findings from a recent study examining NR expression in an independent cohort of normal breast tissue and breast tumors of varying stage [73]. In this study, 41 NRs were detectable via TaqMan low-density array across breast tissues, with 6 of the 7 undetected NRs also not found to be expressed by our criteria in TCGA samples (NR0B1/DAX1, NR0B2/SHP, HNF4A, NR2E1/TLX, NR1H4/FXR and NR5A1/SF1). The only discrepancy between detectible NRs in breast tissue between the former study and our TCGA analysis was the detectible expression of NR1I3/CAR in TCGA samples, which had amongst the lowest expression of NRs in breast tumors in our analysis. Also in validation of our analysis, this study found a general pan-repression of NRs in breast tumors relative to normal breast tissues, with almost half of detected NRs having significantly lower expression.

The RoadMap Epigenome [113] genomics consortia have underscored the significance of NR enhancer interactions. Specifically, the Roadmap Epigenome investigators developed an algorithm entitled `ChromHMM` based on Hidden Markov Models. This was applied to the ∼3000 genomic and epigenomic data sets generated from over 100 cell types to identify 15 different chromatin states [113]. Within these states, enhancer regions [139] represented ∼3% of the genome, and were typified by genomic location, DNAse sensitivity, and histone modifications (e.g., H3K4me1, H3K27me3 and H3K36me3) [140–143]. Within the enhancer modules over 1500 transcription factor motifs were examined for enrichment and revealed 84 significant transcription factor- enhancer modules. Ten of these modules were centered on NRs. Therefore NR are over-represented, and the current study has revealed roles for five of these NRs (*GR*, *GCN1*, *LRH-1*, *THRB*, *RARG*) as being more altered than predicted by chance across thousands of tumor samples. These different NR-motif relationships were not identified in the same chromatin states and therefore they perhaps represent high priority receptors for ChIP-Seq based studies to define how loss of expression (but not deletion or mutation) alters the distribution within chromatin states and modulates enhancer associations and/or responses.

Furthermore, in parallel, large scale genome-wide association studies (GWAS) of genetic variation has revealed that the vast majority of SNPs are contained in areas of the genome that are outside of gene exons, and therefore do not have the potential to make a direct contribution to protein structure and function [144]. It is emerging that many phenotype- and disease-associated SNPs at distal regions impact transcription factor activity that in turn is associated with disease [144, 145], and therefore integration of frequently altered NRs, in the most parsimonious cancer phenotypes, may prove to be a powerful approach to reveal how genetic variation impacts NR function.

## Acknowledgement

This work was supported by the Prostate program of the Department of Defense Congressionally Directed Medical Research Programs [W81XWH-14-1-0608, W81XWH-11-2- 0033 to MJC], the European Union-United States Atlantis Program [P116J090011 to MJC] and in part by National Institute of Health [R01 CA095367-06 and 2R01-CA-095045-06 to MJC]. It was also supported, in part, of the National Cancer Institute Cancer Center Support Grant to the Roswell Park Cancer Institute [CA016056]. It was also supported by the Molecular Pharmacology and Experimental Therapeutics NRSA T32 program [T32CA009072 to MDL].

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