Chapter

Investigating the Role of Mineralocorticoid Receptor Signaling in Cancer Biology in the Genomic Era

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Abstract

In the last decades, advances that take place in the next-generation sequencing and bioinformatics research have helped reveal tissue- and cancer-specific gene expression patterns and mutation landscapes. Indeed, such data are now easily accessible via online genome browsers and different types and levels of public data compendia. Appropriate use of these tools eventually can lead to better patient stratification for diagnosis, prognosis, and therapy of cancers. Mineralocorticoid receptor (MR), encoded by NR3C2 gene, has long been implicated in the development and progression of multiple cancers. Nevertheless, MR has remained relatively understudied at the genomic and transcriptomic levels. In this review, we present the current, literature-based state of knowledge on the role of MR primarily in epithelial cancers. At the same time, we summarize the gene expression, mutation, and copy number variation data on MR obtained from The Cancer Genome Atlas (TCGA). We also show that MR expression could be a promising prognostic marker in different cancers using online tools for survival data analysis. Accordingly, this review strongly demonstrates the emerging potential of studying MR using available tools from the genomics/transcriptomics field for improving cancer diagnosis and prognostication.

Keywords: mineralocorticoid receptor, aldosterone, epithelial cancers, genomics, transcriptomics, prognosis, The Cancer Genome Atlas, www.cbioportal.org

1. Introduction

MR/NR3C2 belongs to the steroid receptor family and it adopts important roles in human physiology and pathology. Although MR has long been studied in renal and cardiovascular contexts, identification of MR in multiple epithelial cancers and presence of cross talk between steroid receptors in cancer-related processes make MR a promising candidate for cancer diagnosis and prognosis. Nevertheless, a focused yet comprehensive literature review about MR's expression in cancers and established role in cancer-associated hallmarks is lacking. Our literature search reveals that MR is expressed in cancerous as well as adjacent and/or normal tissue although the expression of MR can become deregulated during cancer development. Moreover, we provide an account of changes in ligand-dependent or -independent

MR signaling in association with cell proliferation, apoptosis, and senescence of cancer cells. We also identify future directions that can help target novel aspects of MR signaling for mechanistic studies as well as cancer therapeutics. In addition, we point out an emergent need for analyzing the range of genomic alterations and variability in MR expression and its potential association with prognosis across epithelial solid tumors using the existing genomic and transcriptomic resources. We exemplify the extent of variability in MR expression within and among patients based on the patient data found in The Cancer Genome Atlas (TCGA) [1]. Moreover, we demonstrate the profound potential of MR expression as a biomarker for cancer prognostication, i.e., estimation of the likelihood of developing future risks for cancer over a time period using TCGA datasets [2].

2. A concise literature review on MR in cancer biology

Herein an overview of the scientific literature on MR is provided using examples mainly from cancers of epithelial origin including the lung, colon, liver, kidney, pancreas, prostate, breast, and adrenal gland, revealing the understudied aspects of MR in the context of cancer biology.

2.1 Lung cancer

The presence of MR protein in lung cancer tissues has opened new avenues for MR research. Suzuki et al. [3] demonstrated by immunohistochemistry (IHC) that primary lung cancer tissues expressed MR protein along with HSD11B2 enzyme, required for MR receptor specificity through conversion of cortisol to cortisone. However, MR and HSD11B2 proteins, although present and significantly correlated with each other in lung adenocarcinomas, were non-existent in squamous cell, small cell, or large-cell carcinomas [3]. Next, Jeong and colleagues [4] studied gene expression signatures of all 48 nuclear receptors (NRs) including MR in non-small-cell lung cancers (NSCLCs) and corresponding normal lung tissues and found that short heterodimer partner (SHP) and progesterone receptor (PGR) predicted survival in patients with early-stage lung tumors. In the same study, the prognostic role of NRs was also investigated in corresponding normal tissues; and higher expressions of MR and nerve growth factor-induced gene B3, NGFIB3, were identified as predictors of good prognosis for survival and disease recurrence [4]. Furthermore, increasing aldosterone levels in the presence of VEGF inhibitors also proved to be a better indicator of prognosis in NSCLC patients [5]. However, future epidemiological as well as mechanistic studies are needed to address the cross talk between antiangiogenic drugs and MR signaling in lung cancer.

2.2 Colorectal cancer

In colon cancer, an observed decrease in the expression of MR in cancerous tissue in comparison to the adjacent normal mucosa has attributed MR a tumor suppressive role [6]. Tiberio et al. [7] further investigated how MR expression correlated with patient survival in colorectal carcinomas. In this study, the expressions of MR and tumor microvessel density marker protein CD34 were evaluated in tumor and normal colorectal mucosa by IHC, and an inverse correlation of expression was detected between them in colorectal cancers. Kaplan–Meier survival analysis has led to a conclusion that MR could be a tumor suppressor whose decreased expression is correlating well with poor patient survival based on a relatively small number of patients [7]. Recently, spironolactone, an MR antagonist, has also arisen

as a tumor suppressor in colon carcinoma yet independent of MR and through RXRγ receptor signaling [8]. Moreover, recent studies showed that HSD11B2 inhibition, and hence potential dysregulation of MR signaling, could modify gut microbiota, known to be an important factor in colon carcinogenesis [9]. A better understanding of MR cross talk with other nuclear receptors and interaction with gut microbiota from patients treated with MR antagonists is needed in the future.

2.3 Breast cancer

MR expression in normal and diseased breast tissues was initially identified in the 1990s [10, 11]. MR and HSD11B2 proteins were shown to co-localize predominantly in the duct epithelia and to exhibit higher expressions in invasive ductal carcinoma than invasive lobular carcinoma [11]. More recently a study by Conde et al. [12] found that MR expression was peculiar to the cytoplasm of benign and cancerous breast lesions, whereas GR/NR3C1 expression was nuclear in benign breast lesions but showed cytoplasmic as well as nuclear distribution in cancer tissues. These findings might suggest a potential deregulation of GR signaling in malignant tissues, while the effects of MR on tumor development could be less ligand-dependent and/or ligand-insensitive in breast cancer. However, this remains to be assessed.

Induction of growth of lobulo-alveolar structures in mouse mammary gland by MR ligand aldosterone also pointed to the importance of MR signaling in breast biology [13–15]. Furthermore, the presence of progesterone, a potential MR ligand, in the breast tissue highlighted the significance of MR in breast cancers [16]. In addition, in vitro culturing of breast cancer cell lines provided further opportunities for understanding the impact of MR signaling in tumor growth. For example, in the breast cancer cell line PMC42 with detectable MR and HSD11B2 expression levels, aldosterone, alone, did not have an effect on cell proliferation yet when given together with the anti-mineralocorticoid spironolactone resulted in a significant decrease in cell numbers [17]. In another study, aldosterone and cortisol exerted progesterone-like effects such as induction of focal adhesion and reduced cell growth in the progesterone receptor-transfected MDA-MB-231 breast cancer cell line [18]. Recently, genomic and non-genomic actions of aldosterone through G-coupled estrogen receptor (GPER) and MR signaling pathways were demonstrated [19]. Rapid aldosterone exposure activated EGFR and ERK1/2 transduction pathway through MR and GPER in the HER2+ breast cancer cell line SkBr3 and breast tumor-derived endothelial cells [19]. Furthermore, direct interactions among GPER and MR as well as GPER and EGFR were shown, while a long-term exposure to aldosterone increased cell growth which could be inhibited by the silencing of MR expression [19]. These findings indicated possible contributions of GPER activity in the MR-dependent aldosterone signaling and EGFR activation in the regulation of cancer cell growth. However, the relationship between aldosterone and other modulators of GPER, e.g., estrogen, remains to be investigated in breast cancer. MR receptors can exhibit affinity to aldosterone and cortisol as well as other potential ligands likely to be found in the milieu of breast cancer, and thus studying MR, GR, GPER, and/or other receptor crosstalk could be important for better evaluating mammary gland physiology and pathology.

2.4 Liver cancer

As in the lung and colon cancers, MR expression was shown to be downregulated in a large cohort of liver cancer patient tissues [20]. Furthermore, in the same study, overexpression of MR suppressed cancer progression by inhibition of proliferation

and induction of cell cycle arrest eventually leading to apoptosis. Aldosterone's effect on tumor growth was also tested, and its antiproliferative and apoptotic effects were reversed by spironolactone. All of these significantly implicated an evidence for decreased MR signaling in liver cancer pathogenesis [20]. Additionally, this study showed that MR suppressed the Warburg effect [20] by which cancer cells gain growth advantage over normal cells [21] leading to novel insights about MR signaling through cancer research.

Apart from cancer, fibrosis is another significant pathology of the liver. Fibrosis, the leading factor of liver carcinogenesis, occurs due to the accumulation of fibrogenic cells and extracellular matrix (ECM) proteins in excess [22, 23]. ECM is central to sustain cellular homeostasis and integrity, and deregulation of ECM is considered as a hallmark of cancer [24]. The role of aldosterone on ECM synthesis and potentially on liver fibrosis was previously shown in rats, although independent of MR itself [25]. On the other hand, spironolactone was shown to act as a potent provocateur of liver regeneration following partial hepatectomy [26]. As a result, these studies demonstrate the current need for better understanding the role of ligand-dependent and ligand-independent signaling of MR in different facets of liver pathologies including fibrosis, regeneration, and carcinoma.

2.5 Renal cancer

MR and aldosterone signaling have been extensively studied in kidney physiology and pathology for decades [27, 28] and to some degree, in renal carcinomas. Initially, the protein expressions of MR and HSD11B2 were characterized in a large cohort of renal cell neoplasms of different cellular origins using IHC. In this study, co-expression HSD11B2 and MR was shown in normal distal nephron, in chromophobe renal cell carcinoma (chRCC) and oncocytoma of distal nephron origin [29]. In renal cell carcinoma, aldosterone led to upregulation of KRAS oncogene (KRAS4A splice variant) resulting in increased survival and cell proliferation [30]. Yet another study revealed that aldosterone exerted its migratory/metastatic actions through G-protein-coupled estrogen receptor (GPER) in a murine renal cortical adenocarcinoma cell line and also in mice in vivo [31]. These findings implicated important oncogenic pathways and their crosstalk with MR and aldosterone signaling in renal cancers.

Hypertension is a well-established risk factor in kidney cancers potentially due to the genotoxic nature of aldosterone [32, 33]. Indeed, supraphysiological levels of aldosterone treatment induced DNA breaks and chromosomal aberrations in epithelial porcine kidney cells, whereas MR blockade by antagonists prevented formation of such aberrations [34]. Genotoxic effects of aldosterone were investigated further in the DOCA-salt-treated rat model used for inducing MR-dependent hypertension. DOCA-salt treatment caused inflammation, oxidative stress, DNA damage, and increased kidney cell proliferation [35]. Another study highlighted aldosterone-dependent induction of oxidative stress and DNA damage as well as activation of MR-dependent NFKB signaling pathway in kidney tubule cells [36]. Queisser et al. [37] further addressed the downstream signaling pathways triggered by aldosterone-induced oxidative stress both in vitro (porcine kidney cells with proximal tubular properties) and in vivo (rat kidneys). In these models, aldosterone treatment resulted in MR-dependent activation of ERK1/2 and its target, STAT3; and hence aldosterone exposure led to higher proliferation rates while diminishing apoptosis [37]. Accordingly, the role of aldosterone-induced MR signaling in deregulation of DNA damage response needs to be studied also in other epithelial cancers in more detail.

Senescence, evasion of which is another hallmark of cancer [38], was studied in the context of aldosterone signaling in different renal models. For example, $p16^{INK4a}$, a cyclin-dependent kinase inhibitor and a cellular senescence marker [39, 40], was induced in the kidneys and hearts of DOCA-salt-treated rats [41]. These effects could be reversed by antihypertensives and spironolactone, suggesting a potential role of MR signaling in the regulation of the senescent phenotype [41]. In another study, senescence was investigated in aldosterone-infused rats and cultured human proximal tubular cells. In both models, aldosterone-induced senescence-like characteristics were marked by senescence-associated beta-galactosidase staining, p21/Cdkn1a and p53 overexpression, and SIRT1 under-expression. MR blockade either using eplerenone (in vivo) or through gene silencing (in vitro) sufficiently reversed the aldosterone-induced senescence-like characteristics [42]. In line with this study, Kitada et al. [43] also showed the presence of aldosterone-induced senescence, characterized by increased p21 expression and beta-galactosidase staining, in human proximal tubular cells. Furthermore, a prolonged exposure to aldosterone triggered p21-mediated cytokine release, e.g., TNF alpha, which in turn led to apoptosis [43]. All of these have implicated MR signaling through interaction with aldosterone in the induction of senescence, an inherent autoregulatory mechanism of proliferating cells with established tumor suppressive activity. The role of MR/aldosterone-induced senescence in epithelial cancers however needs to be further studied since this can provide an effective route for therapeutic invention.

2.6 Pancreas cancer

Recently, MR has been ascribed a tumor suppressive role also in pancreatic ductal adenocarcinoma (PDAC) [44]. In PDAC patients, dysregulated expression of macrophage migration inhibitory factor (MIF) was associated with disease aggressiveness, and MIF-driven upregulation of miR-301b was shown to suppress MR expression [44]. In turn, MR expression resulted in the inhibition of epithelial to mesenchymal transition (EMT) and increased chemotherapeutic drug (gemcitabine) sensitivity. Consistently, survival data analysis further associated downregulation of MR expression with poor survival in PDAC patients [44]. However, PDAC remains one of the cancers receiving less attention in the MR field; future studies can address the role of genomic and non-genomic effects of MR signaling in PDAC.

2.7 Prostate cancer

Detection of 11 beta-hydroxysteroid dehydrogenase enzyme [45] and a functional MR in the androgen-dependent prostate cancer cell line LNCaP cells dates back to the early 1990s [46]. More recently, Dovio et al. [47] assessed GR and MR expression together with HSD11B-1 and HSD11B-2 enzyme activity upon inflammatory stimulus (IL1B stimulation) or basal conditions in the androgen-dependent and androgen-independent prostate cancer cell lines. Diverse expression patterns of MR, GR, and HSD11B enzyme activities were detected among cell lines, while downstream effects of IL1B exposure were inhibited by cortisol or dexamethasone in a cell line-dependent manner [47].

Another lead for the role of mineralocorticoids in prostate cancer has come from abiraterone acetate (AA), an androgen synthesis inhibitor, used for metastatic castration-resistant prostate cancer (mCRPC), which results in secondary mineralocorticoid excess [48]. Androgen-induced conformational changes in androgen

receptor (AR) could be inhibited in the presence of mineralocorticoids (corticosterone or deoxycorticosterone). However, administration of corticosterone alone resulted in repression of AR transcriptional activity and cellular growth at concentrations present in the serum of AA-administered patients [49]. Pia et al. [50] focusing on identifying ways of eliminating adrenocorticotropic hormone (ACTH)-dependent AA-induced mineralocorticoid excess showed that a low effective dose of glucocorticoid together with MR antagonist and salt deprivation could be an ideal treatment. Indeed, the use of prednisone, a synthetic glucocorticoid, could overcome the effects of secondary mineralocorticoid excess in mCRPC patients treated with abiraterone [51]. Effect of eplerenone-abiraterone co-administration on secondary mineralocorticoid excess syndrome and progression-free survival (PFS) was evaluated and compared to prednisone-abiraterone co-administered in patients. No significant difference was obtained by means of mineralocorticoid excess syndrome characteristics and PFS between these two experimental groups; and this has raised AA-eplerenone as an alternative therapy for overcoming prednisone-induced side effects [52]. Enzalutamide is another antiandrogen drug used for treating metastatic prostate cancer patients; however, resistance gained against enzalutamide therapy remains a challenge. GR signaling induces antiandrogen resistance by hijacking AR function [53, 54]; hence, the therapeutic effects of enzalutamide-corticosteroid co-administration were addressed in prostate cancer cells [55]. Dexamethasone decreased the therapeutic effects of enzalutamide as well as increasing resistance. However, prednisolone and aldosterone diminished resistance to enzalutamide. Consistently, silencing of MR resulted in enhanced resistance to enzalutamide and AR activity [55]. Moreover, the effects of diverse antihypertensive medication on prostate cancer survival following radical prostatectomy were tested in the Finnish population, and overall, the antihypertensive treatment was associated with increased death risk [56]. These findings clearly establish the importance of AR and MR cross talk and complex ligand interactions in prostate cancer, which could be further studied in other cancers and cancer subtypes where AR signaling can play a role.

2.8 Adrenocortical cancer

Adrenal incidentalomas include adrenocortical adenomas, adrenocortical carcinomas, and pheochromocytoma [57, 58]. Aldosterone-producing adenomas (APA, i.e., benign tumors of the adrenal glands) account for 35% of the diseases of the primary aldosteronism spectrum [59, 60]. Somatic mutations occurring in KCNJ5, CACNA1D, ATP1A1, ATP2B3, and CTNNB1 genes give rise to sporadic APA [61]. In addition to the abovementioned mutations, regulatory RNAs such as miRNAs have also been shown to be important in modulating aldosterone levels and tumorigenesis [62–64]. On the other hand, adrenocortical cancers (ACC), some of which are hormone-producing, occur relatively rarely, and patients with ACC exhibit poor prognosis with a median survival of 5.5 years [65]. In the adrenocortical cell line H295R, aldosterone in vitro is shown to upregulate T-type calcium channel expression and currents, an effect reversed by spironolactone [66]. H295R cells have also been shown to express components of aldosterone signaling pathway including ENaC subunits, NEDD4L, SGK1, MR, and HSD11B2 [66, 67]. Although several factors, such as age, resection margin and proliferation scores, uterine steroid profiles, CpG island hypermethylation status, as well as levels of selected biomarkers, have been tested for their contribution in ACC prognosis, the importance of MR expression status is yet to be evaluated in ACC patients [65, 68, 69]. Future studies should investigate the role of activation/inactivation of MR-aldosterone signaling in diagnosis/prognosis of ACC for which significant

amounts of genomic and transcriptomic data have recently become available (please see the next section for details).

3. Cancer genome and transcriptome analyses for MR using online tools

The advent of whole-genome sequencing and development and availability of genome browsers, such as UCSC genome browser [70, 71] and Ensembl [72, 73], in the early 2000s have enabled researchers to identify genes/genomic intervals that are important in human physiology and pathology. This is mainly done by association of genome fragments with informative tracks that range from expression values to the presence of copy number and single nucleotide variations (CNVs and SNVs, respectively). Cataloging and annotation of the human genome with regard to genomic and transcriptomic variation have also revolutionized cancer research [74, 75]. The NIH-driven giant effort named The Cancer Genome Atlas (TCGA) has published its first results in 2008 on the genomic and transcriptomic landscape of gliomas [76]. Over the years, TCGA has expanded to house thousands of cancer genomes/transcriptomes/proteomes from tens of different cancers allowing researchers from all over the world to have unlimited access to cancer-related datasets [1]. Many different web-based tools nowadays use TCGA and Catalog of Somatic Mutations in Cancer (COSMIC) [77] as primary resources and build on them to extract data from user-provided queries and/or to perform gene-specific or genome-wide secondary analyses, such as cbioportal.org [78, 79]. These webservers make use of a wide range of information and incorporate quantitative and statistical analyses and visualization tools and help users with little or no programming experience perform cancer bioinformatics analyses.

The expression of MR along with those of other nuclear receptors has been studied in a recent TCGA PanCancer transcriptomics study of bladder, breast, colon, head and neck, liver, and prostate cancers; and MR expression is shown to be downregulated in all [80]. To demonstrate the potential of TCGA in revealing the importance of MR in cancer research, we have used www.cbioportal.org webserver [78, 79] to visualize the next-generation RNA sequencing data from different cancers of TCGA provisional datasets for MR and showed that MR mRNA is expressed differentially across many tumor types (**Figure 1**). Among these, chromophobe renal cell carcinoma (chRCC) has the highest expression of MR followed by thyroid carcinoma (THCA), pheochromocytoma and paragangliomas (PCPG), and adrenocortical carcinoma (ACC), while carcinomas of the bladder, breast, cervix, esophagus, head, and neck exhibit high variability (**Figure 1**). It is also apparent that genomic alterations (gains and shallow deletions) are common in many of these TCGA dataset patients (**Figure 1**). Future analyses can focus on how these alterations are associated with MR expression in different cancers, especially in chRCC and ACC.

Overall, the observed rate of somatic mutations of MR has been shown to be significantly lower with respect to the expected rate suggesting MR does not tolerate well mutations with functional constraints [81]. On the other hand, functional mutations of MR have been identified in different contexts including renal pseudohypoaldosteronism as well as hypertension [81, 82]. In addition, relatively different residues in the DNA-binding domain of MR seem to be affected between type I pseudohypoaldosteronism and cancers [83]. Herein we have compiled the MR mutation landscape for cancers found in TCGA provisional datasets (cbioportal.org) showing the number (95 missense, 19 truncating totaling 0.9% somatic mutation frequency) as well as the distribution of mutations across the MR protein sequence (984 amino acids long) and the DNA-binding domain zinc finger, C4 type

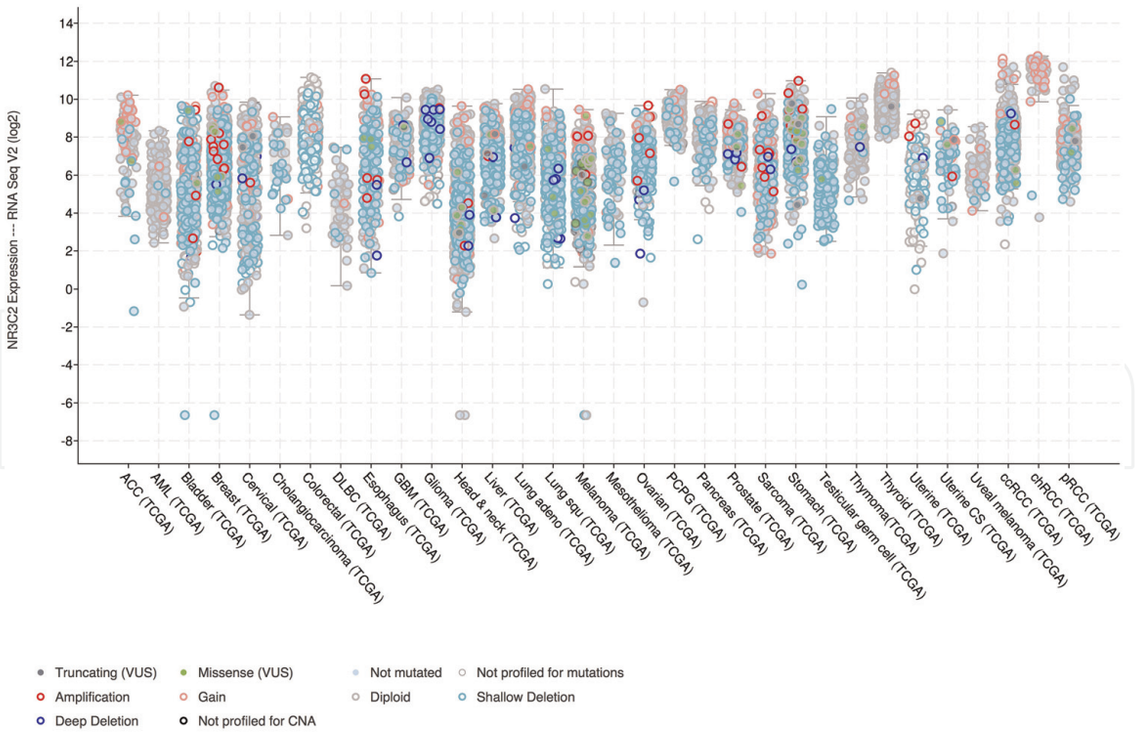

Figure 1.
Boxplots of MR/NR3C2 expression across different TCGA provisional datasets obtained from cbioportal.org.

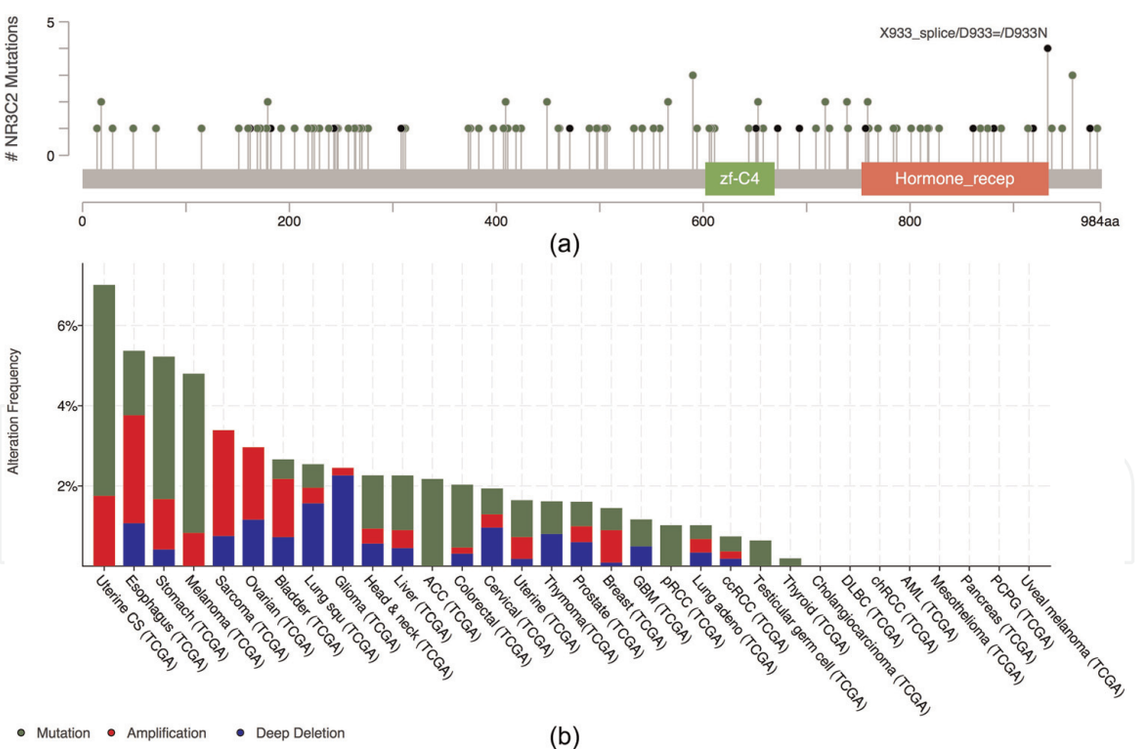

Figure 2.
Mutation landscape and distribution of genetic alterations in MR. (a) Schema showing the location of MR mutations and the two functional domains of MR. (b) The bar graph showing the percentages of mutations, amplifications, and deletions in TCGA provisional datasets. Source: cbioportal.org.

(zf-C4; 602–669), and ligand-binding domain of NR (753–934) (**Figure 2A**). However, it is important to note that TCGA datasets are dynamic in nature such that new samples as well as mutation/CNV annotations are continually being added. www.cbioportal.org webserver also offers two separate and large multi-cancer

Sample ID	Cancer Type	Amino Acid	MutationAssessor	SIFT	Polyphen-2
TCGA-32-2494-01	Glioblastoma Multiforme	C606W	high	deleterious	probably_damaging
TCGA-QK-A8Z8-01	Head and Neck Squamous Cell Carcinoma	C658G	high	deleterious	probably_damaging
TCGA-D3-A8GI-06	Cutaneous Melanoma	M668I	high	deleterious	probably_damaging
TCGA-G3-A25W-01	Hepatocellular Carcinoma	C658F	high	deleterious	probably_damaging
coadread_dfci_2016_3235	Colorectal Adenocarcinoma	F626L	high	deleterious	probably_damaging
MM-0308	Plasma Cell Myeloma	F626C	high	deleterious	probably_damaging

Table 1.
High-impact MR mutations based on MutationAssessor in curated TCGA and non-TCGA dataset collection (www.cbioportal.org).

collections, i.e., TCGA PanCancer and curated TCGA/non-TCGA (curated set of non-redundant) datasets. Upon analysis of these two collections, the numbers of observed missense mutations in the MR gene increase to 193 and 281, and truncating mutations to 34 and 48, respectively. It is also possible to download functional annotations for these mutations that include scores showing the impact of mutations, e.g., analyzed through MutationAssessor.org [84, 85]. Accordingly, in the curated TCGA/non-TCGA dataset collection, we have identified, among all 334 mutations, 6 high-impact mutations, all of which are located in the zf-C4 domain (**Table 1**). Functional analysis of MR mutation landscape thus can help researchers select high-impact variants for future validation studies using MutationAssessor as well as other tools linked with www.cbioportal.org, e.g., SIFT [86] and PolyPhen-2 [87].

Moreover, using TCGA provisional datasets, we analyzed whether MR accumulated different rates of genetic alterations in different cancers (**Figure 2B**). The mean proportion of genetic alterations per cancer was 0.024 (0.017–0.031, 95% confidence interval, CI). The results in percentages showed that around 7% of uterine carcinosarcoma patients exhibited genomic alterations (mutation, amplification; 7.02% in 57 cases), while the second and third ranking cancers were those of esophageal carcinoma (5.38% in 186 cases) and stomach adenocarcinomas (5.23% in 478 cases) (**Figure 2B**). As mentioned above the ranking of these cancers in terms of percent genomic alterations can be dynamic depending on which data collection has been used. For example, when using TCGA PanCancer sample collection, which reports a more complete mutation/CNV annotation information, the first ranking cancer with the highest percentage of genetic alterations has become melanoma (8.71% out of 448 skin cutaneous melanoma) followed by uterine carcinomas (7.75% out of 529 uterine corpus endometrial carcinoma and 7.02% out of 57 uterine carcinosarcomas). Future studies may focus on uterine carcinomas and melanoma to address the mechanisms and effects of these observed alterations.

4. Investigating the role of MR in cancer patient stratification and prognosis using genomics resources

Survival analysis is often used for studying the association of an event of interest, e.g., death and disease recurrence, with another clinical or biological variable [88]. Analysis of TCGA-associated survival data (overall survival (OS) and/or relapse-free survival (RFS)) is available through several online webservers including GEPIA [89], OncoLnc [90], Kaplan–Meier Plotter [91], and KM-Express [92]. These tools help evaluate survival of cancer patients whose genomic/transcriptomic and clinical data are stored in TCGA, by using Cox coefficient statistics, hazard ratio (HR) and/or logrank tests, and Kaplan–Meier plots. GEPIA, which has previously

been used in prognostic identification of several biomarkers in ACC [93, 94], performs survival statistics in addition to providing other functionalities such as co-expression analysis and diagnostic marker prediction. We analyzed data from all cancers available in GEPIA and showed that an NR3C2/MR expression higher than the median level predicted a significantly better prognosis (OS) and low HR in adrenocortical carcinoma (ACC), kidney renal clear cell carcinoma (KIRC), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), brain lower-grade glioma (LGG), and liver hepatocellular carcinoma (LIHC) (logrank p-value <0.1; **Figure 3**).

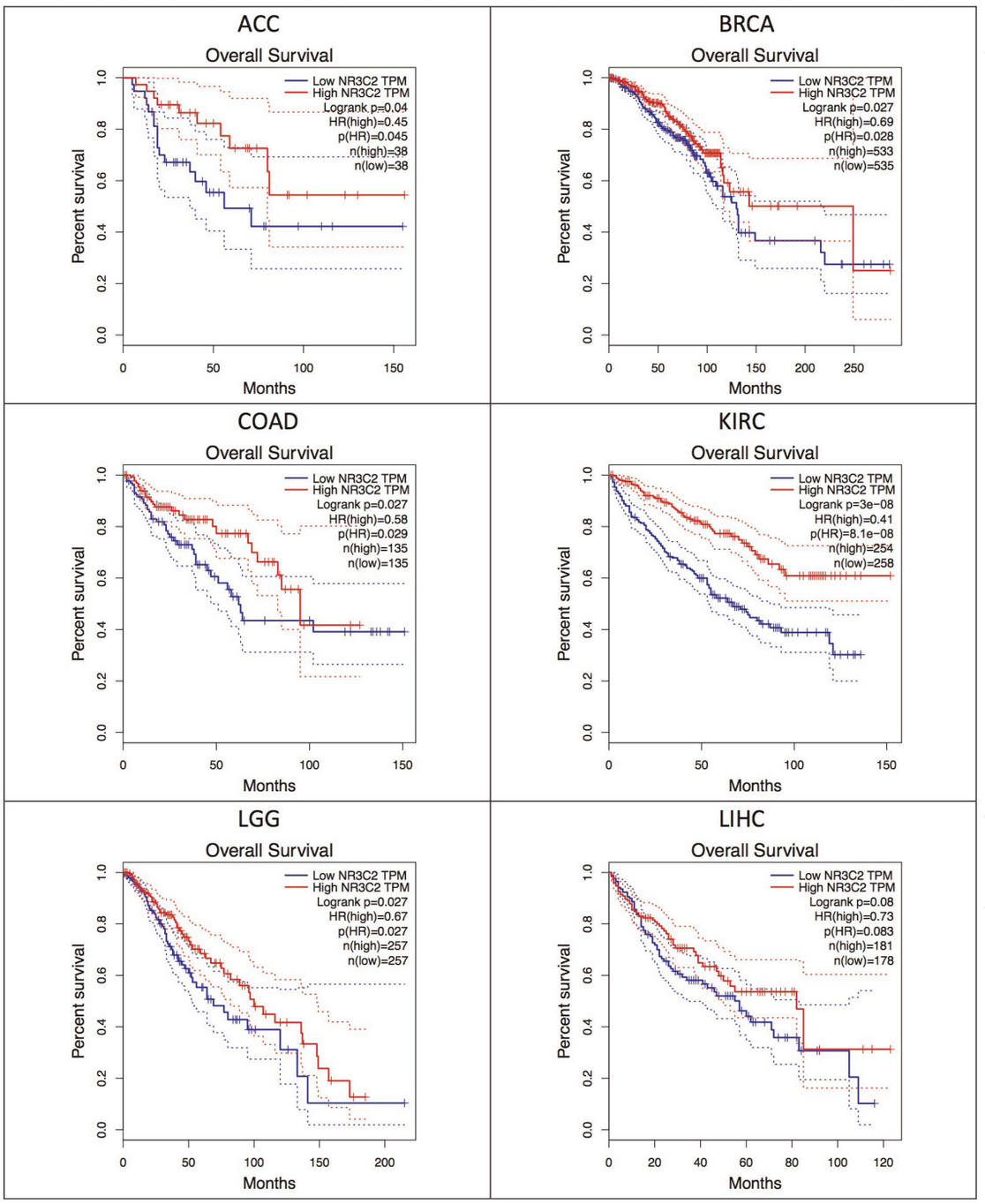

Figure 3.
MR expression-based overall survival (OS) analyses performed using GEPIA. Adrenocortical carcinoma (ACC), breast invasive carcinoma (BRCA), colorectal adenocarcinoma (COAD), kidney renal clear cell carcinoma, low-grade glioma (LGG), and liver hepatocellular carcinoma (LIHC). The statistics and their associated p-values are shown on graphs, while groups (red and blue) are separated by the median expression level of MR.

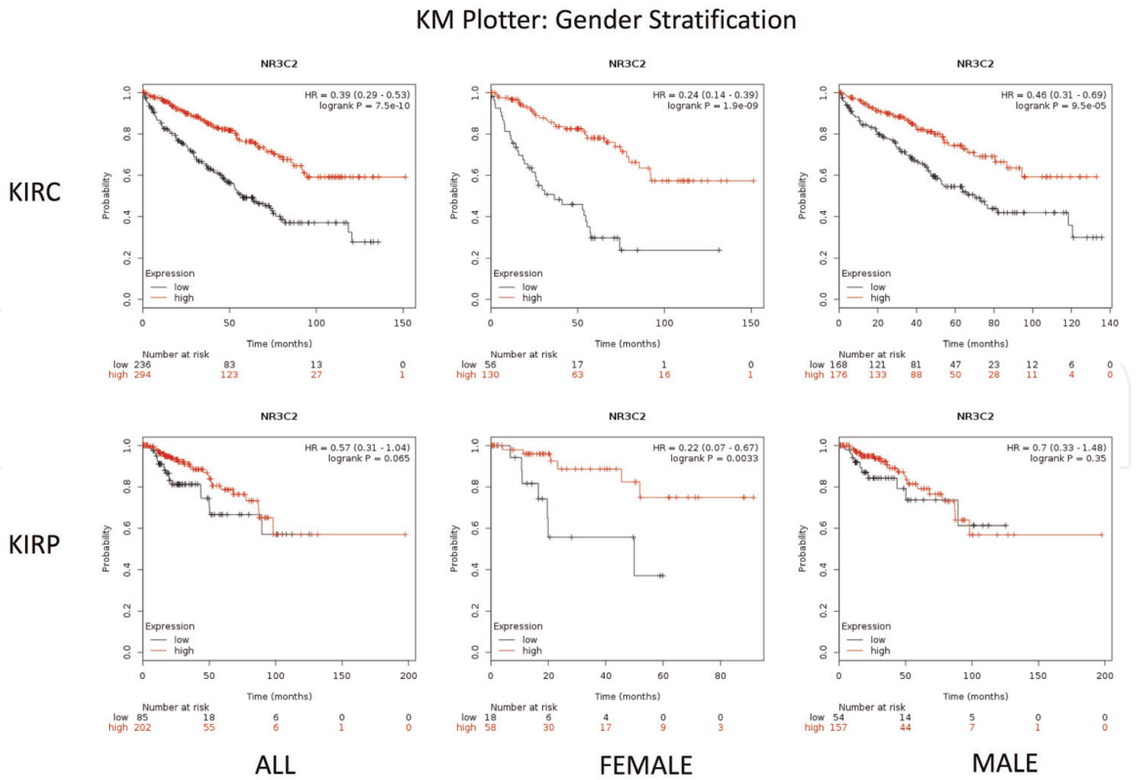

Figure 4.
KM plotter analysis of kidney renal clear cell carcinoma (KIRC) and kidney renal papillary cell carcinoma (KIRP) for all, female and male patients, separately.

Similar analyses can be performed using other webservers, such as KM plotter (http://kmplot.com/analysis/), which allows for auto-selection of an expression threshold that performs best in the logrank test. Recently, KM plotter has also made possible the stratification of TCGA PanCancer patient data according to different clinical and demographic variables including gender [95]. For example, we tested the significance of association between MR expression and OS using the best cutoff option separately for females and males, in renal cancers, KIRC and KIRP. Accordingly, we found that sexual dimorphism in MR expression can play a role in association with OS, warranting further investigation (**Figure 4**). Importantly, it is also possible to study MR expression and its role in RFS using the same tools. Our findings through GEPIA and KM plotter help confirm that downregulation of MR/NR3C2 expression can be significantly associated with cancer progress as has been previously reported in the literature. In conclusion, large-scale expression analysis in association with clinical data such as time to death or recurrence can thus reveal the importance of MR expression in epithelial and other solid tumors yet warrants further mechanistic studies.

5. Conclusion

A comprehensive look at the history of MR in cancer research strongly implicates the dysregulation of MR signaling in the development and progression of epithelial cancers. However, the interactions with its natural ligand aldosterone and/or with other potential ligands, such as tissue-specific progesterone as well as the growing evidence on the presence of receptor cross talk, complicate the "tumor suppressive" role often attributed to MR. MR's relatively well-established effects in renal tissue senescence, oxidative stress and DNA damage, as well as its emerging

potential in the regulation of Warburg effect and fibrosis/regeneration in liver tissue represent novel avenues to pursue especially in the context of cancer therapy. In addition, the genome-wide availability of CNV, SNV, and mRNA expression profiles from cancer patients enables comparisons within and between different tumors providing an enhanced level of accessibility to researchers in the field of cancer biology. Indeed, online examination of the interaction between different data sources such as expression and patient survival data is now effectively possible for MR and can be extended to other genes participating in MR signaling. Generation of large-scale genome-wide data along with the development of tools that help analyze and integrate such data is likely to further enhance our understanding of MR in the development and progression of different cancers.

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References

[1] Weinstein JN, Collisson EA, Mills GB, Shaw KRM, Ozenberger BA, Ellrott K, et al. The cancer genome atlas pan-cancer analysis project. Nature Genetics. 2013;**45**:1113-1120. DOI: 10.1038/NG.2764

[2] Moons KGM, Royston P, Vergouwe Y, Grobbee DE, Altman DG. Prognosis and prognostic research: What, why, and how? British Medical Journal. 2009; **338**:b375. DOI: 10.1136/BMJ.B375

[3] Suzuki S, Suzuki T, Tsubochi H, Koike K, Tateno H, Krozowski ZS, et al. Expression of 11 beta-hydroxysteroid dehydrogenase type 2 and mineralocorticoid receptor in primary lung carcinomas. Anticancer Research. 2000;**20**:323-328

[4] Jeong Y, Xie Y, Xiao G, Behrens C, Girard L, Wistuba II, et al. Nuclear receptor expression defines a set of prognostic biomarkers for lung cancer. PLoS Medicine. 2010;**7**: e1000378. DOI: 10.1371/JOURNAL.PMED.1000378

[5] Bar J, Ding K, Zhao H, Han L, Laurie SA, Seymour L, et al. Angiotensin-converting enzyme and aldosterone serum levels as prognostic and predictive biomarkers for cediranib in NCIC clinical trials group study BR.24. Clinical Lung Cancer. 2015;**16**: e189-e201. DOI: 10.1016/J.CLLC.2015.05.002

[6] Di Fabio F, Alvarado C, Majdan A, Gologan A, Voda L, Mitmaker E, et al. Underexpression of mineralocorticoid receptor in colorectal carcinomas and association with VEGFR-2 overexpression. Journal of Gastrointestinal Surgery. 2007; **11**:1521-1528

[7] Tiberio L, Nascimbeni R, Villanacci V, Casella C, Fra A, Vezzoli V, et al. The decrease of mineralcorticoid receptor drives angiogenic pathways in colorectal cancer. PLoS One. 2013;**8**:e59410. DOI: 10.1371/JOURNAL.PONE.0059410

[8] Leung W-H, Vong QP, Lin W, Janke L, Chen T, Leung W. Modulation of NKG2D ligand expression and metastasis in tumors by spironolactone via RXRγ activation. The Journal of Experimental Medicine. 2013;**210**: 2675-2692. DOI: 10.1084/JEM.20122292

[9] Morris DJ, Ridlon JM. Glucocorticoids and gut bacteria: "The GALF hypothesis" in the metagenomic era. Steroids. 2017;**125**:1-13. DOI: 10.1016/J.STEROIDS.2017.06.002

[10] Sasano H, Fukushima K, Sasaki I, Matsuno S, Nagura H, Krozowski ZS. Immunolocalization of mineralocorticoid receptor in human kidney, pancreas, salivary, mammary and sweat glands: A light and electron microscopic immunohistochemical study. Journal of Endocrinology. 1992; **132**:305-310. DOI: 10.1677/JOE.0.1320305

[11] Sasano H, Frost AR, Saitohi R, Matsunaga G, Nagura H, Krozowski ZS, et al. Localization of mineralocorticoid receptor and 11 beta-hydroxysteroid dehydrogenase type II in human breast and its disorders. Anticancer Research. 1997;**17**:2001-2007

[12] Conde I, Paniagua R, Fraile B, Lucio J, Arenas MI. Glucocorticoid receptor changes its cellular location with breast cancer development. Histology and Histopathology. 2008;**23**:77-85

[13] Ichinose RR, Nandi S. Influence of hormones on lobulo-alveolar differentiation of mouse mammary glands in vitro. The Journal of Endocrinology. 1966;**35**:331-340

[14] Wood BG, Washburn LL, Mukherjee AS, Banerjee MR. Hormonal regulation of lobulo alveolar growth,

functional differentiation and regression of whole mouse mammary gland in organ culture. The Journal of Endocrinology. 1975;**65**:1-6

[15] Hussain-Hakimjee EA, Peng X, Mehta RR, Mehta RG. Growth inhibition of carcinogen-transformed MCF-12F breast epithelial cells and hormone-sensitive BT-474 breast cancer cells by 1α-hydroxyvitamin D5. Carcinogenesis. 2006;**27**:551-559. DOI: 10.1093/CARCIN/BGI231

[16] Moore NL, Hickey TE, Butler LM, Tilley WD. Multiple nuclear receptor signaling pathways mediate the actions of synthetic progestins in target cells. Molecular and Cellular Endocrinology. 2012;**357**:60-70. DOI: 10.1016/J.MCE.2011.09.019

[17] Koyama K, Myles K, Smith R, Krozowski Z. Expression of the 11beta-hydroxysteroid dehydrogenase type II enzyme in breast tumors and modulation of activity and cell growth in PMC42 cells. Journal of Steroid Biochemistry and Molecular Biology. 2001;**76**:153-159. DOI: 10.1016/S0960-0760(00)00157-6

[18] Leo JC, Guo C, Woon CT, Aw SE, Lin VC. Glucocorticoid and mineralocorticoid cross-talk with progesterone receptor to induce focal adhesion and growth inhibition in breast cancer cells. Endocrinology. 2004;**145**:1314-1321

[19] Rigiracciolo DC, Scarpelli A, Lappano R, Pisano A, Santolla MF, Avino S, et al. GPER is involved in the stimulatory effects of aldosterone in breast cancer cells and breast tumor-derived endothelial cells. Oncotarget. 2016;**7**:94-111. DOI: 10.18632/ONCOTARGET.6475

[20] Nie H, Li J, Yang XM, Cao QZ, Feng MX, Xue F, et al. Mineralocorticoid receptor suppresses cancer progression and the Warburg effect by modulating the miR-338-3p-PKLR axis in hepatocellular carcinoma. Hepatology. 2015;**62**:1145-1159. DOI: 10.1002/HEP.27940

[21] Vander HMG, Cantley LC, Thompson CB. Understanding the Warburg effect: The metabolic requirements of cell proliferation. Science. 2009;**324**:1029-1033. DOI: 10.1126/SCIENCE.1160809

[22] Aydin MM, Akcali KC. Liver fibrosis. Turkish Journal of Gastroenterology. 2018;**29**:14-21. DOI: 10.5152/TJG.2018.18330

[23] Bataller R, Brenner DA. Liver fibrosis. The Journal of Clinical Investigation. 2005;**115**:209-218. DOI: 10.1042/BJ20071570

[24] Cox TR, Erler JT. Remodelling of the extracellular matrix: Implications for cancer. In: Schatten H, editor. Cell and Molecular Biology of Breast Cancer. New York: Springer; 2013. pp. 65-90. DOI: 10.1007/978-1-62703-634-4_4

[25] Rombouts K, Niki T, Wielant A, Hellemans K, Schuppan D, Kormoss N, et al. Effect of aldosterone on collagen steady state levels in primary and subcultured rat hepatic stellate cells. Journal of Hepatology. 2001;**34**:230-238. DOI: 10.1016/S0168-8278(00)00087-8

[26] Parlakgumus A, Colakoglu T, Kayaselcuk F, Colakoglu S, Ezer A, Calskan K, et al. Two drugs with paradoxical effects on liver regeneration through antiangiogenesis and antifibrosis: Losartan and spironolactone: A pharmacologic dilemma on hepatocyte proliferation. Journal of Surgical Research. 2013;**179**:60-65. DOI: 10.1016/J.JSS.2012.08.046

[27] Fuller PJ, Funder JW. Mineralocorticoid and glucocorticoid receptors in human kidney. Kidney International. 1976;**10**:154-157

[28] Matulich DT, Spindler BJ, Schambelan M, Baxter JD. Mineralocorticoid receptors in human kidney. The Journal of Clinical Endocrinology and Metabolism. 1976; **43**:1170-1174

[29] Yakirevich E, Morris DJ, Tavares R, Meitner PA, Lechpammer M, Noble L, et al. Mineralocorticoid receptor and 11β-hydroxysteroid dehydrogenase type II expression in renal cell neoplasms: A tissue microarray and quantitative RT-PCR study. American Journal of Surgical Pathology. 2008;**32**:874-883. DOI: 10.1097/PAS.0B013E31815F2362

[30] King S, Bray S, Galbraith S, Christie L, Fleming S. Evidence for aldosterone-dependent growth of renal cell carcinoma. International Journal of Experimental Pathology. 2014;**95**: 244-250. DOI: 10.1111/IEP.12074

[31] Feldman RD, Ding Q, Hussain Y, Limbird LE, Pickering JG, Gros R. Aldosterone mediates metastatic spread of renal cancer via the G protein-coupled estrogen receptor (GPER). FASEB Journal. 2016;**30**:2086-2096. DOI: 10.1096/FJ.15-275552

[32] Weikert S, Boeing H, Pischon T, Weikert C, Olsen A, Tjonneland A, et al. Blood pressure and risk of renal cell carcinoma in the European prospective investigation into cancer and nutrition. American Journal of Epidemiology. 2008;**167**:438-446. DOI: 10.1093/AJE/KWM321

[33] Sanfilippo KM, McTigue KM, Fidler CJ, Neaton JD, Chang Y, Fried LF, et al. Hypertension and obesity and the risk of kidney cancer in 2 large cohorts of US men and women. Hypertension. 2014;**63**:934-941. DOI: 10.1161/HYPERTENSIONAHA.113.02953

[34] Schupp N, Queisser N, Wolf M, Kolkhof P, Bärfacker L, Schäfer S, et al. Aldosterone causes DNA strand breaks and chromosomal damage in renal cells, which are prevented by mineralocorticoid receptor antagonists. Hormone and Metabolic Research. 2010;**42**:458-465. DOI: 10.1055/S-0029-1243253

[35] Schupp N, Kolkhof P, Queisser N, Gärtner S, Schmid U, Kretschmer A, et al. Mineralocorticoid receptor-mediated DNA damage in kidneys of DOCA-salt hypertensive rats. The FASEB Journal. 2011;**25**:968-978. DOI: 10.1096/FJ.10-173286

[36] Queisser N, Oteiza PI, Stopper H, Oli RG, Schupp N. Aldosterone induces oxidative stress, oxidative DNA damage and NF-KB-activation in kidney tubule cells. Molecular Carcinogenesis. 2011;**50**:123-135. DOI: 10.1002/MC.20710

[37] Queisser N, Schupp N, Schwarz E, Hartmann C, Mackenzie GG, Oteiza PI. Aldosterone activates the oncogenic signals ERK1/2 and STAT3 via redox-regulated mechanisms. Molecular Carcinogenesis. 2017;**56**:1868-1883. DOI: 10.1002/MC.22643

[38] Dimri GP. What has senescence got to do with cancer? Cancer Cell. 2005;**7**: 505-512. DOI: 10.1016/J.CCR.2005.05.025

[39] Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16(INK4a). Cell. 1997;**88**:593-602. DOI: 10.1016/S0092-8674(00)81902-9

[40] Alcorta DA, Xiong Y, Phelps D, Hannon G, Beach D, Barrett JC. Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. Proceedings of the National Academy of Sciences. 1996;**93**: 13742-13747. DOI: 10.1073/PNAS.93.24.13742

[41] Westhoff JH, Hilgers KF, Steinbach MP, Hartner A, Klanke B, Amann K,

et al. Hypertension induces somatic cellular senescence in rats and humans by induction of cell cycle inhibitor p16INK4a. Hypertension. 2008;**52**:123-129. DOI: 10.1161/HYPERTENSIONAHA.107.099432

[42] Fan YY, Kohno M, Hitomi H, Kitada K, Fujisawa Y, Yatabe J, et al. Aldosterone/mineralocorticoid receptor stimulation induces cellular senescence in the kidney. Endocrinology. 2011;**152**: 680-688. DOI: 10.1210/EN.2010-0829

[43] Kitada K, Nakano D, Hitomi H, Kobori H, Deguchi K, Mori H, et al. Aldosterone induces p21-regulated apoptosis via increased synthesis and secretion of tumour necrosis factor- α in human proximal tubular cells. Clinical and Experimental Pharmacology and Physiology. 2012;**39**:858-863. DOI: 10.1111/J.1440-1681.2012.12001.X

[44] Yang S, He P, Wang J, Schetter A, Tang W, Funamizu N, et al. A novel MIF signaling pathway drives the malignant character of pancreatic cancer by targeting NR3C2. Cancer Research. 2016;**76**:3838-3850. DOI: 10.1158/0008-5472.CAN-15-2841

[45] Nath N, Lakshmi V, Rosenthal JC. Presence of 11 β-hydroxysteroid dehydrogenase enzyme in the human prostate tumor cell line LNCaP. The Prostate. 1993;**23**:225-233. DOI: 10.1002/PROS.2990230305

[46] Pagé N, Warriar N, Govindan MV. 11β-Hydroxysteroid dehydrogenase and tissue specificity of androgen action in human prostate cancer cell LNCaP. The Journal of Steroid Biochemistry and Molecular Biology. 1994;**49**:173-181. DOI: 10.1016/0960-0760(94)90008-6

[47] Dovio A, Sartori M, De Francia S, Mussino S, Perotti P, Saba L, et al. Differential expression of determinants of glucocorticoid sensitivity in androgen-dependent and androgen-independent human prostate cancer cell lines. The Journal of Steroid Biochemistry and Molecular Biology. 2009;**116**:29-36

[48] Attard G, Reid AHM, Yap TA, Raynaud F, Dowsett M, Settatree S, et al. Phase I clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven. Journal of Clinical Oncology. 2008;**26**:4563-4571. DOI: 10.1200/JCO.2007.15.9749

[49] Kim W, Jones JO, Diamond M, Haqq C, Molina A, Small EJ, et al. Inhibition of the androgen receptor by mineralocorticoids at levels physiologically achieved in serum in patients treated with abiraterone acetate. Prostate Cancer and Prostatic Diseases. 2014;**17**:292-299. DOI: 10.1038/PCAN.2014.27

[50] Pia A, Vignani F, Attard G, Tucci M, Bironzo P, Scagliotti G, et al. Strategies for managing ACTH dependent mineralocorticoid excess induced by abiraterone. Cancer Treatment Reviews. 2013;**39**:966-973. DOI: 10.1016/J.CTRV.2013.03.003

[51] Auchus RJ, Yu MK, Nguyen S, Mundle SD. Use of prednisone with abiraterone acetate in metastatic castration-resistant prostate cancer. The Oncologist. 2014;**19**:1231-1240. DOI: 10.1634/THEONCOLOGIST.2014-0167

[52] Gill D, Gaston D, Bailey E, Hahn A, Gupta S, Batten J, et al. Efficacy of eplerenone in the management of mineralocorticoid excess in men with metastatic castration-resistant prostate cancer treated with abiraterone without prednisone. Clinical Genitourinary Cancer. 2017;**15**:e599-e602. DOI: 10.1016/J.CLGC.2016.12.008

[53] Isikbay M, Otto K, Kregel S, Kach J, Cai Y, Vander Griend DJ, et al. Glucocorticoid receptor activity contributes to resistance to

androgen-targeted therapy in prostate cancer. Hormones and Cancer. 2014;**5**: 72-89. DOI: 10.1007/S12672-014-0173-2

[54] Arora VK, Schenkein E, Murali R, Subudhi SK, Wongvipat J, Balbas MD, et al. Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. Cell. 2013;**155**:1309-1322. DOI: 10.1016/J.CELL.2013.11.012

[55] Shiota M, Fujimoto N, Higashijima K, Imada K, Kashiwagi E, Takeuchi A, et al. Mineralocorticoid receptor signaling affects therapeutic effect of enzalutamide. The Prostate. 2018;**78**: 1045-1052. DOI: 10.1002/PROS.23661

[56] Santala EE, Rannikko A, Murtola TJ. Antihypertensive drugs and prostate cancer survival after radical prostatectomy in Finland—A nationwide cohort study. International Journal of Cancer. 2018;**144**:440-447. DOI: 10.1002/IJC.31802

[57] Terzolo M, Bovio S, Pia A, Reimondo G, Angeli A. Management of adrenal incidentaloma. Best Practice & Research: Clinical Endocrinology & Metabolism. 2009;**23**:233-243. DOI: 10.1016/J.BEEM.2009.04.001

[58] Crona J, Backman S, Welin S, Taïeb D, Hellman P, Stålberg P, et al. RNA-sequencing analysis of adrenocortical carcinoma, pheochromocytoma and paraganglioma from a pan-cancer perspective. Cancers. 2018;**10**:518. DOI: 10.3390/CANCERS10120518

[59] Conn JW, Knopf RF, Nesbit RM. Clinical characteristics of primary aldosteronism from an analysis of 145 cases. The American Journal of Surgery. 1964;**107**:159-172. DOI: 10.1016/0002-9610(64)90252-1

[60] Young WF. Primary aldosteronism: Renaissance of a syndrome. Clinical Endocrinology. 2007;**66**:607-618. DOI: 10.1111/J.1365-2265.2007.02775.X

[61] El Zein RM, Boulkroun S, Fernandes-Rosa FL, Zennaro MC. Molecular genetics of Conn adenomas in the era of exome analysis. La Presse Médicale. 2018;**47**:e151-e158. DOI: 10.1016/J.LPM.2018.07.006

[62] Peng KY, Chang HM, Lin YF, Chan CK, Chang CH, Chueh SCJ, et al. MiRNA-203 modulates aldosterone levels and cell proliferation by targeting wnt5a in aldosterone-producing adenomas. The Journal of Clinical Endocrinology and Metabolism. 2018; **103**:3737-3747. DOI: 10.1210/JC.2018-00746

[63] Zhang G, Zou X, Liu Q, Xie T, Huang R, Kang H, et al. MiR-193a-3p functions as a tumour suppressor in human aldosterone-producing adrenocortical adenoma by down-regulating CYP11B2. International Journal of Experimental Pathology. 2018;**99**:77-86. DOI: 10.1111/IEP.12267

[64] He J, Cao Y, Su T, Jiang Y, Jiang L, Zhou W, et al. Downregulation of miR-375 in aldosterone-producing adenomas promotes tumour cell growth via MTDH. Clinical Endocrinology. 2015;**83**: 581-589. DOI: 10.1111/CEN.12814

[65] Calissendorff J, Calissendorff F, Falhammar H. Adrenocortical cancer: Mortality, hormone secretion, proliferation and urine steroids—Experience from a single centre spanning three decades. BMC Endocrine Disorders. 2016;**16**:15. DOI: 10.1186/S12902-016-0095-9

[66] Lesouhaitier O, Chiappe A, Rossier MF. Aldosterone increases T-type calcium currents in human adrenocarcinoma (H295R) cells by inducing channel expression. Endocrinology. 2001;**142**:4320-4330. DOI: 10.1210/ENDO.142.10.8435

[67] Burton TJ, Cope G, Wang J, Sim JC, Azizan EAB, O'Shaughnessy KM, et al. Expression of the epithelial Na^+ channel

and other components of an aldosterone response pathway in human adrenocortical cells. European Journal of Pharmacology. 2009;**613**:176-181. DOI: 10.1016/J.EJPHAR.2009.04.005

[68] Mohan DR, Lerario AM, Else T, Mukherjee B, Almeida MQ, Vinco M, et al. Targeted assessment of G0S2 methylation identifies a rapidly recurrent, routinely fatal molecular subtype of adrenocortical carcinoma. Clinical Cancer Research. 2019;**25**:3276-3288. DOI: 10.1158/1078-0432.CCR-18-2693

[69] Liu S, Ding G, Zhou Z, Feng C. β-Catenin-driven adrenocortical carcinoma is characterized with immune exclusion. OncoTargets and Therapy. 2018;**11**:2029-2036. DOI: 10.2147/OTT.S159979

[70] James Kent W, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, et al. The human genome browser at UCSC. Genome Research. 2002;**12**: 996-1006. DOI: 10.1101/GR.229102

[71] Karolchik D, Baertsch R, Diekhans M, Furey TS, Hinrichs A, Lu YT, et al. The UCSC genome browser database. Nucleic Acids Research. 2003;**31**:51-54. DOI: 10.1093/NAR/GKG129

[72] Hubbard T. The Ensembl genome database project. Nucleic Acids Research. 2002;**30**:38-41. DOI: 10.1093/NAR/30.1.38

[73] Stalker J, Gibbins B, Meidl P, Smith J, Spooner W, Hotz HR, et al. The Ensembl web site: Mechanics of a genome browser. Genome Research. 2004;**14**:951-955. DOI: 10.1101/GR.1863004

[74] Hede K. Superhighway or blind alley? The cancer genome atlas releases first results. Journal of the National Cancer Institute. 2008;**100**:1566-1569. DOI: 10.1093/JNCI/DJN424

[75] Mardis ER, Wilson RK. Cancer genome sequencing: A review. Human Molecular Genetics. 2009;**18**:R163-R168. DOI: 10.1093/HMG/DDP396

[76] McLendon R, Friedman A, Bigner D, Van Meir EG, Brat DJ, Mastrogianakis GM, et al. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008;**455**:1061-1068. DOI: 10.1038/NATURE07385

[77] Forbes SA, Bhamra G, Bamford S, Dawson E, Kok C, Clements J, et al. The catalogue of somatic mutations in cancer (COSMIC). Current Protocols in Human Genetics. 2008;**57**:10.11.1-10.11.26. DOI:10.1002/0471142905.HG1011S57

[78] Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. Cancer Discovery. 2012;**2**:401-404. DOI: 10.1158/2159-8290.CD-12-0095

[79] Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Science Signaling. 2013;**6**: pl1. DOI: 10.1016/J.EXPNEUROL.2012.04.021

[80] Long MD, Campbell MJ. Pan-cancer analyses of the nuclear receptor superfamily. Nuclear Receptor Research. 2015;**2**:101182. DOI: 10.11131/2015/101182

[81] Zennaro MC, Fernandes-Rosa F. Mineralocorticoid receptor mutations. Journal of Endocrinology. 2017;**234**:T93-T106. DOI: 10.1530/JOE-17-0089

[82] Mohaupt MG, Frey FJ. Mineralocorticoid receptor malfunction: Further insights from rare forms of hypertension. Nephrology Dialysis Transplantation. 2002;**17**:539-542. DOI: 10.1093/NDT/17.4.539

[83] Hudson WH, Youn C, Ortlund EA. Crystal structure of the mineralocorticoid receptor DNA binding domain in complex with DNA. PLoS One. 2014;**9**:e107000. DOI: 10.1371/JOURNAL.PONE.0107000

[84] Reva B, Antipin Y, Sander C. Determinants of protein function revealed by combinatorial entropy optimization. Genome Biology. 2007;**8**: R232. DOI: 10.1186/GB-2007-8-11-R232

[85] Reva B, Antipin Y, Sander C. Predicting the functional impact of protein mutations: Application to cancer genomics. Nucleic Acids Research. 2011; **39**:e118. DOI: 10.1093/NAR/GKR407

[86] Sim NL, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: Predicting effects of amino acid substitutions on proteins. Nucleic Acids Research. 2012;**40**:W452-W457. DOI: 10.1093/NAR/GKS539

[87] Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Current Protocols in Human Genetics. 2013;**76**: 7.20.1-7.20.41. DOI: 10.1002/ 0471142905.HG0720S76

[88] Gray RJ. Flexible methods for analyzing survival data using splines, with applications to breast cancer prognosis. Journal of the American Statistical Association. 1992;**87**:942-951. DOI: 10.1080/01621459.1992.10476248

[89] Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Research. 2017;**45**: W98-W102. DOI: 10.1093/NAR/ GKX247

[90] Anaya J. OncoLnc: Linking TCGA survival data to mRNAs, miRNAs, and lncRNAs. PeerJ Computer Science. 2016;**2**:e67. DOI: 10.7717/PEERJ-CS.67

[91] Lánczky A, Nagy Á, Bottai G, Munkácsy G, Szabó A, Santarpia L, et al. miRpower: A web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients. Breast Cancer Research and Treatment. 2016;**160**:439-446. DOI: 10.1007/S10549-016-4013-7

[92] Chen X, Miao Z, DIvate M, Zhao Z, Cheung E. KM-express: An integrated online patient survival and gene expression analysis tool for the identification and functional characterization of prognostic markers in breast and prostate cancers. Database. 2018;**2018**:bay069. DOI: 10.1093/ DATABASE/BAY069

[93] Xiao H, Xu D, Chen P, Zeng G, Wang X, Zhang X. Identification of five genes as a potential biomarker for predicting Progress and prognosis in adrenocortical carcinoma. Journal of Cancer. 2018;**9**:4484-4495. DOI: 10.7150/JCA.26698

[94] Yuan L, Qian G, Chen L, Wu CL, Dan HC, Xiao Y, et al. Co-expression network analysis of biomarkers for adrenocortical carcinoma. Frontiers in Genetics. 2018;**9**:328. DOI: 10.3389/ FGENE.2018.00328

[95] Nagy Á, Lánczky A, Menyhárt O, Gyorffy B. Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets. Scientific Reports. 2018;**8**:9227. DOI: 10.1038/ S41598-018-27521-Y