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Chapter

Transcriptional Regulation and Epigenetics in Cardiovascular Cells: Role of the Mineralocorticoid Receptor

Lisa Deng, Lutz Hein and Achim Lother

Abstract

The mineralocorticoid receptor (MR), a ligand-activated transcription factor, plays an important role in the pathophysiology of cardiovascular disease. Epigenetic mechanisms such as DNA methylation or histone modifications in addition to the DNA sequence are decisive regulators of cell type-specific transcriptional activity and gene expression by controlling chromatin accessibility. In this review, we summarise the current knowledge about the impact of MR on gene expression in cardiovascular cells. We discuss studies investigating the interaction of MR with epigenetic mechanisms or other transcription factors and their implications for the cardiovascular system. Finally, we compare mechanisms of transcriptional regulation by MR and other nuclear transcription factors. In conclusion, MR is an important regulator of gene expression in cardiovascular cells. Potential mechanisms of cell type-specific transcriptional regulation by MR include interaction with other transcription factors or co-regulators, tethering and post-translational modifications of the MR. Further studies will be needed to clarify the interplay of MR and epigenetic mechanisms.

Keywords: mineralocorticoid receptor, epigenetics, chromatin, gene expression, cell types

1. Introduction

Mineralocorticoid receptor (MR) antagonists are a cornerstone in the current pharmacological therapy of chronic heart failure [1, 2]. MR in renal epithelial cells plays an important role for ion homeostasis and blood pressure control, but MR is also expressed in extrarenal tissue including different cell types of the heart and the vasculature [3]. This understanding has subsequently triggered intense research on the molecular basis of MR activity in the cardiovascular system. The MR is a member of the nuclear receptor (NR) family, which consists of different DNA-binding transcription factors [4]. As a ligand-activated transcription factor, MR controls the expression of its target genes. Despite increasing evidence for rapid, non-genomic effects of MR, regulation of gene expression is still regarded as a key feature of MR action. The MR holds a unique position among steroid-activated nuclear receptors as it binds two ligands, aldosterone as the main ligand as well

as cortisol (corticosterone in rodents) with similar affinity. Indeed, progesterone which acts as an antagonist at the human MR [5] and, according to recent studies, has also activating properties at chicken and zebrafish MR [6] could also be seen as a third ligand to the MR. Despite their diverse range of action, nuclear receptors share a common, highly conserved structure [7]. A ligand-induced conformational change of the ligand-binding domain (LDB) leads to the activation of the receptor. Upon translocation into the nucleus, NR modulates transcription by interacting with specific DNA sequences. Eukaryote DNA is not linear but wrapped up in a complex with histones forming the chromatin. Histones are subjected to a variety of reversible post-translational modifications, which, in turn, can change chromatin density, accessibility and high-order chromatin structure and thereby determine transcription factor binding and gene expression [8].

Epigenetics is an emerging topic in cardiovascular research. The heart and the vasculature are composed of numerous different cell types including cardiac myocytes, endothelial cells, vascular smooth muscle cells, fibroblasts, immune cells and others [9]. These different cell types show marked heterogeneity in their specific transcriptomes [10–13]. Transcriptome analyses of different cell types of the heart revealed transcriptional changes during development and disease [12, 13], and these changes were associated with distinct alterations in the epigenome. For example, dysregulation of histone modifications or DNA methylation has been linked to numerous diseases of the cardiovascular system, such as atherosclerosis [14–16], hypertension [17, 18] or heart failure [19–22]. Based on these findings pharmacological targeting of epigenetic modifiers has been proposed for treatment of cardiovascular disease and successfully tested in preclinical models [23, 24].

Epigenetic modifications, such as histone modifications or DNA methylation, have been identified as decisive regulators of transcription factor activity, as chromatin compaction determines the cell-specific accessibility of DNA [4]. Vice versa, NR can alter the chromatin structure through recruitment of cofactors, which act as chromatin remodelers [25]. Chromatin immunoprecipitation (ChIP) assays with subsequent deep sequencing represent a powerful technique for genome-wide analysis of histone modifications or other DNA-associated proteins [26]. The combination of different histone modifications, often referred to as the histone code, gives information on proximal and distal regulatory DNA elements, including promoters and enhancers. Promoters are often located in close proximity to the transcription start site (TSS) and associated with monomethylation of histone 3 at lysin 4 (H3K4me1), while enhancers are usually distal to TSS and marked by trimethylation of H3K4 (H3K4me3). Active promoter or enhancer sites are marked by acetylation of histone 3 at lysin 27 (H3K27ac); in contrast, H3K9me3 and H3K27me3 mark heterochromatic or repressed regions [27]. Epigenetic modifications are reversible, being recognised, established or removed by reader, writer and eraser proteins. For example, acetylation of histones is regulated by two counteracting enzymes which add (histone acetyltransferases, HAT) acetyl groups to lysine residues or remove them (histone deacetylases HDAC) [28].

DNA methylation of CpGs is the only known epigenetic mechanism directly targeting the DNA and plays an important role in gene silencing when being recognised by reader proteins such as methyl-CpG-binding protein 2 (MeCP2) that act as transcriptional repressors [29]. DNA methylation is established and maintained during mitosis by DNA methyltransferases (DNMT) [29]. Oxidation of methylcytosines to hydroxylmethylcytosines, which is an intermediate step towards demethylation, is mediated by members of the ten-eleven translocation (TET) enzyme family. This occurs predominantly in promoter and enhancer regions with low CpG density, resulting in low methylated regions and can be used to identify active regulatory regions [29, 30]. CpG-rich regions, also referred to as CpG islands,

in the promoter of constitutively active genes are typically unmethylated [29]. This allows to use DNA methylation as a stable mark of cell lineage during development [31]. Analysis of the DNA methylome of murine cardiomyocytes revealed distinct DNA methylation pattern during cardiomyocyte development and disease [32]. Demethylation of cardiac gene bodies correlated with active histone marks and increased gene expression. Interestingly, by comparison of the DNA methylome of healthy and failing cardiomyocytes, the DNA methylation pattern of failing cardiomyocytes was bearing a partial resemblance to foetal cardiomyocytes; however, changes were not major [32]. A similar result could be observed in human failing cardiac myocytes. Pathological gene expression in heart failure was accompanied by changes in active histone marks, whereas the DNA methylation pattern remained mostly the same [33]. Similarly, changes in the transcriptome of cardiac myocytes following myocardial infarction were accompanied by altered accessibility of chromatin [12].

Given the important role of epigenetics in transcriptional regulation, interaction of MR with epigenetic modifications; with epigenetic reader, writer or eraser proteins; or with other transcription factors might control the cell type-specific impact of MR on gene expression and function. In this review article, we will summarise what is known about MR-dependent gene expression, epigenetic mechanisms and their interaction with MR in cardiovascular cells.

2. Impact of aldosterone and MR on gene expression in the cardiovascular system

A series of experimental studies during the past years revealed distinct MR functions in cardiac myocytes, fibroblasts, endothelial cells, vascular smooth muscle cells and immune cells [3, 34, 35]. These studies provided evidence that the beneficial effect of MR antagonists in heart failure is directly related to MR in cardiovascular cells and independent from MR in renal epithelial cells. Several attempts have been made to understand the downstream signalling events following MR activation and to identify direct MR target genes in different cells or tissues of the cardiovascular system.

Early studies on cultured fibroblasts revealed an upregulation of collagen by aldosterone [36, 37] and by this suggested MR-dependent gene expression in cardiovascular cells. Later studies applied microarray or RNA-sequencing techniques to systematically detect MR-responsive genes. Similar to fibroblasts, aldosterone induced the expression of collagen types I and III in cultured smooth muscle cells from the coronary artery [38]. In mouse aorta, aldosterone regulated the expression of genes related to vascular function, such as oxidative stress, extracellular matrix and angiogenesis [39]. Treatment of EAhy926 endothelial cells expressing MR after retroviral transfection with aldosterone leads to regulation of only 17 transcripts [40]. In contrast, 133 genes were found up- or downregulated by aldosterone in human umbilical vein endothelial cells with naïve MR expression but not after MR knockdown [41]. These genes were associated leukocyte migration and angiogenesis. Interestingly, aldosterone treatment had opposing effects on endothelial cell gene expression when compared to treatment with vascular endothelial cell growth factor, a potent pro-angiogenic factor [41].

In a H9C2 cardiac myocyte cell line stably expressing MR, 53 transcripts were detected to be differentially regulated after treatment with aldosterone, the majority of them being upregulated [42]. Among the upregulated transcripts were genes related to extracellular matrix deposition such as *Adamts1* (A disintegrin and metalloprotease with thrombospondin motifs), *Pai-1* (plasminogen-activator inhibitor 1)

or *Tnx* (tenascin-X) [42]. Studies using selective MR or GR antagonists confirmed MR-dependent expression of these genes in H9C2 cells [43, 44]. In heart tissue from untreated mice overexpressing MR in cardiac myocytes, microarray analysis revealed 24 transcripts upregulated and 22 transcripts downregulated. Again, the expression of *Adamts1* and *Pai-1* was induced by MR overexpression [43]. Vice versa, MR deletion from cardiac myocytes leads to differential regulation of 158 genes in heart tissue, including upregulation of *Nppa* (atrial natriuretic peptide type A); however, there was no clear reduction of genes related to collagen synthesis in this study [45]. In doxorubicin-treated mice, MR deletion from cardiac myocytes prevented the repressive effect of doxorubicin on gene expression, likely by a post-transcriptional mechanism [46]. One well-investigated gene that is upregulated in heart tissue by cardiac myocyte MR overexpression or aldosterone treatment is neutrophil-gelatinase-associated lipocalin (*Ngal*) [47]. Interestingly, *Ngal* was upregulated by aldosterone in endothelial cells and vascular smooth muscle cells as well [47].

Taken together, there is an overlap of several genes that were similarly regulated by MR in different cardiovascular cells or tissues including *Sgk1* [41–43, 45], *Tsc22d3* [40, 41], *Adamts1* [41–43], *Fkbp5* [40, 41, 43], *Klf9* [39–41], *Ngal* [47] or *Per1* [41, 48, 49], indicating a common signature of MR-regulated genes. Interestingly, these genes are well-related to the pathophysiological impact of MR on fibrosis and inflammation in the cardiovascular system.

3. Regulation of MR transcriptional activity

Transcriptional activity of MR is regulated at different levels: ligand binding, nuclear translocation, chromatin state and MR-DNA interaction. The main focus of this article will be on chromatin state and MR-DNA interaction; however, some specificities of cardiovascular cell types should be noticed: First, as MR binds to aldosterone or glucocorticoids with similar affinity, different mechanisms exist that allow ligand-specificity of MR. One of them is co-expression of 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2), an enzyme converting glucocorticoids to derivates that are inactive at the MR (for review, see [50]). In the cardiovascular system, 11βHSD2 is highly expressed in endothelial cells, low expressed in cardiac myocytes and smooth muscle cells and probably absent in immune cells [51]. Second, while in most cell types unliganded MR is predominantly located at the cytoplasm and shuttles into the nucleus upon ligand binding, MR has been described to be constitutively located at the nucleus in cardiac myocytes [52]. Immunohistochemical analysis of heart tissue revealed nuclear localization of the MR, and subfractional analysis showed that the vast majority of MR was chromatin-bound irrespective of plasma aldosterone or glucocorticoid levels. The subcellular distribution depended on the balance of heat shock protein 90 expression and the synergy of two nuclear localization signals. Interestingly, transcriptional activation of chromatin-bound MR nevertheless required the presence of a ligand [52].

4. Validation of MR-responsive genes

To further elucidate the role of MR in transcriptional regulation, it has been aimed to identify genes that are direct MR targets by proofing MR binding to a corresponding regulatory region by ChIP experiments. In a human embryonic kidney cell line (HEK293) stably transfected with myc-tagged hMR, *Cnksr3* was identified as a novel MR target gene containing MR binding sites (MBS) upstream

of the transcription start site using ChIP in conjunction with microarray analysis (ChIP-chip). *Cnksr3* was described as highly expressed in response to aldosterone, mediating ENaC activity and thereby influencing transepithelial Na^+ transport in renal collecting duct cells [53]. Ueda et al. combined ChIP with high-throughput sequencing for a genome-wide analysis of MR target genes in renal distal convoluted tubular cells and identified 1113 MR binding sites associated with 1414 genes [54]. Combining data from a microarray study 186 genes were considered to be aldosterone-responsive, showing an increase in mRNA expression levels after aldosterone stimulation and a decrease in expression by inhibitory treatment with spironolactone. Interestingly, only 25 genes showed an overlap between both assays and were thus classified as MR target genes, among those the well-known target genes *Sgk1*, *Fkbp5* and *Tsc22d3* [54]. Of note, strong enrichment of MR does not necessarily translate into increased expression of target genes. In another study, analysis of renal MR target genes revealed four commonly acknowledged target of MR signalling in renal cells such as *Scnn1a*, *Fkbp5*, *Zbtb16* and *Per1* and nine other genes not associated with MR signalling before [55]. MR target genes could be subcategorized according to their different kinetics of MR-dependent activation of gene expression into early, intermediate and late response genes [55]. However, molecular mechanisms of MR signalling are highly cell-specific, as distinct target genes could be found in one experiment but not reproduced in another [54, 55], whereas other MR target genes seem to be independent from tissue or cell type such as *Fkbp5* or *Per1* [54–57].

MR binding sites seem to be widespread across the genome, an equal proportion (almost 40%) of all identified peaks were located either within introns or intergenic. Surprisingly, only 40 of all approximately 1000 MBS were found within promoter regions and 11% within enhancer regions [55]. Among the 13 genes correlating to the highest MR binding peak scores in human renal cells, *LINC00963* was not regulated by MR, despite high aldosterone-induced MR recruitment. As the MR binding site was located far from the nearest TSS, the involvement of this remote MBS in transcriptional regulation of another gene through long-range chromatin interactions is possible [55, 58].

For cardiovascular cells, available studies are restricted to MR binding in the promoter region of certain target genes. $Ca_v1.2$, a voltage-gated calcium channel, could be identified as an MR target in the cardiovascular system using mutational and in silico analysis. An aldosterone-dependent recruitment of MR to the alternative cardiac *Cacna1c* P1-promoter was observed, subsequently regulating expression of cardiac $Ca_v1.2$ transcripts in cardiac myocytes as well as in vascular smooth muscle cells, underlining the importance of aldosterone signalling in vascular reactivity and regulation of blood pressure [59]. In a similar approach, MR-dependent regulation of *Icam1* in cultured endothelial cells was demonstrated using a reporter assay with different promoter fragments [60].

5. Mineralocorticoid receptor response elements

The canonical GRE DNA motif consists of two inverted palindromic half sites, separated by a 3 bp spacer [AGAACAnnnTGTTCT (**Figure 1**)] [61]. Different variations of this motif exist in human renal cells with the bases C5 and G11 remaining essential for MR binding. Strong sequence degeneration of the classic consensus motif correlated with low MR enrichment and therefore disrupted MR interaction [55]. A small percentage of 7.4% MBS contained the classic consensus MRE motif; the larger part of all identified MRE consisted of half sites or a combination out of palindromic sequences and half-MREs [55]. Of note, not all MR binding sites

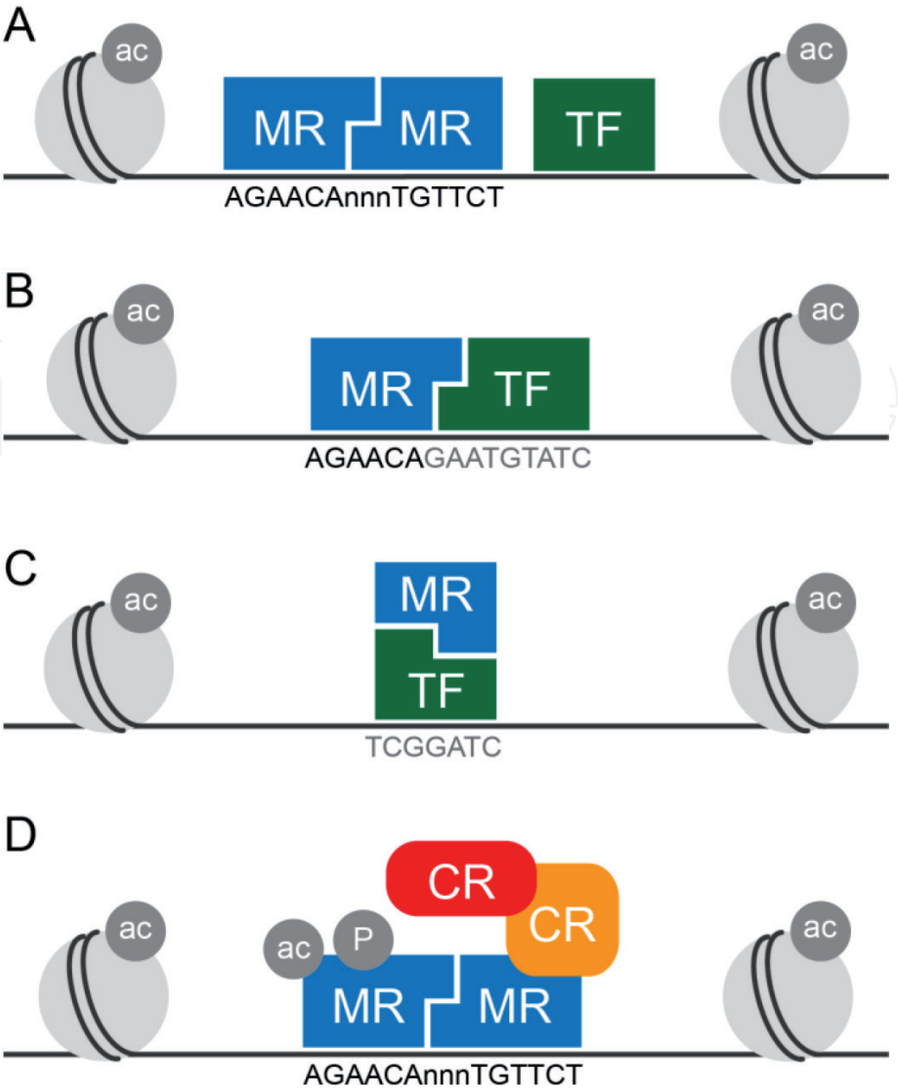

Figure 1.
Proposed modes of MR-DNA interaction. Mineralocorticoid receptors can directly bind to MR response elements consisting of a 15 bp palindromic DNA sequence (A) or to composite elements when interacting with a neighbouring transcription factor (B). Indirect MR-DNA interaction can be facilitated by tethering to another transcription factor (C). MR-DNA interaction is modulated by co-regulatory transcription factors or post-translational modifications of the MR (D). MR, mineralocorticoid receptor; TF, transcription factor; CR, co-regulator; ac, acetylation; P, phosphorylation.

contain an MRE consensus motif. The majority of peaks lacked the MRE implying interaction of MR with the DNA directly by binding to specific DNA sequences different from the consensus motif, indirectly via protein–protein interactions or possibly through tethering as known for GR (**Figure 1**) [55, 62, 63]. Indeed, an aldosterone-dependent *trans*-activation of AP-1 could recently be proven in human cells, underlining this hypothesis [64]. Interestingly, all MBS in rat hippocampal tissue contained an MRE [57], suggesting that the mode of MR-DNA interaction might be cell- or tissue-specific.

When analysing MRE it has to be taken into account that MR and GR share a common consensus sequence. Both receptors are simultaneously expressed in various tissues and interact with each other. For example, MR and GR are able to form homodimers as well as heterodimers in rat hippocampal tissue after acute stress challenges [56]. The binding of homodimers of the respective receptors or MR/GR heterodimers seems to be gene-specific. Using a tandem ChIP approach, it could be pointed out that MR and GR mainly bind as MR/MR and GR/GR homodimers at the GRE of *Sgk1*, whereas MR/GR heterodimer formation could be proven for the

Per1 GRE in addition to homodimers of both receptors. Notably, at the *Fkbp5* GRE MR seems to bind only in a heterocomplex with GR [56], indicating distinct mechanisms controlling binding of receptors to GRE, such as local chromatin accessibility or interactions with co-regulators [65]. Interactions of MR/GR heterodimers with the *Per1* promoter in response to aldosterone or cortisol stimulation could also be detected in a human renal cell line [49]. In an extensive time-course ChIP-qPCR study, the authors provide evidence for different dynamics in MR or GR recruitment to the promoter region of a given target gene. MR recruitment kinetics onto the *Per1* promoter was ligand-dependent. Aldosterone induces a distinct kinetic pattern differing from MR-GR recruitment kinetics [49], suggesting different dynamics as mechanisms for receptor selectivity. MR/GR heterodimer show distinct dissociating rates differing from MR/MR or GR/GR homodimers [66], resulting in a stronger GRE binding and a synergistic effect on controlling transcriptional activity of each receptor [49, 56]. However, contrasting results have been reported. MR/GR heterodimers either enhanced [56] or inhibited [67] transcription of given target genes when compared to respective homodimers. In cardiac myocytes, *Per1* was upregulated by both cortisol and aldosterone. The impact of aldosterone was enhanced in the presence of the CLOCK transcription factor, suggesting a cooperative effect of both transcription factors on *Per1* expression [48]. Meinel et al. demonstrated an interaction of MR with the transcription factor specificity protein 1 (SP1) leading to the binding of an alternative MRE in the promoter region of epidermal growth factor receptor (EGFR) gene. The SP-1-dependent transactivation of EGFR through MR could also be demonstrated in cultured smooth vascular cells [68].

Comparison of GR-ChIP data with MR-ChIP data from rat hippocampal tissue revealed 918 MR-specific binding sites, 1450 GR-specific and 475 binding sites shared by MR and GR, all containing the GRE motif [57]. In all MR-exclusive binding sites, an additional motif, corresponding to the Atoh1 binding sequence, was present, and the protein was not expressed in hippocampal tissue. However, in Atoh1 belonging to the basic helix–loop–helix (bHLH) family of transcription factors, the brain-specific NeuroD family members could be identified as potential candidates for interaction with MR, thus ensuring MR specificity at MR-exclusive binding sites [57]. Different studies point at a role of differences in the nucleotide sequence of GREs in mediating distinct transcriptional activity of MR and GR [56, 59, 68], as some GRE do not enhance binding of the receptors [56], other GRE sequences favouring MR binding [69] or exclusively binding MR [68]. The ability to bind negative glucocorticoid response elements (nGRE) and therefore repressing transcription of given target genes is restricted to GR signalling and not shared with other steroid receptors [70]. Despite having a common ancestral with the capacity of binding nGRE, the ability was lost in the MR due to different mutations at independent timepoints and enhanced in the GR lineage, resulting and contributing to the capability of MR and GR to show specific and distinct transcriptional signatures even though being highly homologous. Nevertheless, repressing effects on transcriptional activities are not unique for GR as MR are likewise able to *trans*-repress NFκB signalling through tethering effects without interfering with DNA binding of the complex, putting an interesting angle to the pro- and antiinflammatory effects of MR and GR. However, *trans*-repressing effects of MR are notably weaker than those of GR [64]. Of note, in addition to the beforementioned activating protein-protein interactions of MR on inflammatory AP-1 signalling, suppressing effects on AP-1 activities in a DNA-sequence-specific manner—and therefore target gene-specific—could also be elucidated, stressing out the necessity of identifying cell type-specific target genes of MR in order to dissect augmenting effects of MR from repressing effects for the development of potential new MR antagonists [64].

6. Co-regulators of MR activity

MR transcriptional activity in the nucleus is finely controlled by a variety of different mechanisms, including the recruitment of co-regulators, a heterogeneous group of non-receptor proteins (**Figure 1**) [71]. Co-regulators modulate the transcriptional activity of the receptor by either acting as coactivators or as corepressors [72]. They predominantly interact at specific regions in the NTD and at the LBD. The NTD represents the least conserved region across the steroid receptor family and thereby harbouring most potential for differential recruitment of co-regulators [73]. The LDB is conserved between different species and harbours a ligand-dependent activation function site (AF-2), which is exposed upon conformational changes induced by ligand binding of the receptor [74]. AF-2 as a docking platform is essential for co-regulator binding. Many co-regulator molecules interact via an LxxLL (L stands for a leucine, x for any other amino acid) motif with the AF-2 region [50], e.g. the first identified and well-characterised NR co-regulator steroid receptor coactivator-1 (SRC-1) [73]. Among the over 400 putative co-regulators discovered in screening assays, a few MR-specific interaction partners could be characterised such as the elongation factor eleven-nineteen lysine-rich leukaemia (ELL). ELL is able to differentially regulate MR and GR, selectively enhancing MR transcriptional activity and repressing GR-mediated transactivation [75]. GEMIN4 represents an MR corepressor, attenuating MR transcriptional activity in a cell- and gene-specific manner, as a repressive effect could be demonstrated in human embryonic kidney cells but not in a rat cardiomyocyte cell line [76]. However, GEMIN4 actions are not restricted to MR, leaving NF-YC as the only described MR-specific corepressor [77]. Just recently, a specific MR cofactor modulation has been proposed as a molecular mechanism for the differential antifibrotic properties of the novel nonsteroidal MR antagonist finerenone when compared to steroidal MR antagonists [78].

7. Post-translational modification of the MR

Post-translational modifications such as phosphorylation, ubiquitination, sumoylation or acetylation are described to influence MR transactivation [79]. Phosphorylation of MR has contrasting effects, as it has reported early that phosphorylation of MR is necessary for aldosterone binding and enhancing the DNA-binding ability of MR [80, 81]. On the other hand, phosphorylation of MR on serine and threonine residues mediated by cyclin-dependent kinase 5 (CDK5) was shown to attenuate MR transcriptional activity, whereas nuclear receptor accumulation was not altered, suggesting impaired interaction of MR with co-regulators [82]. Recently, the ubiquitously expressed casein kinase 2 (CK2) was identified as a positive modulator of MR transcriptional activity by direct phosphorylation of the receptor and potentially by changing the phosphorylation status of other MR-co-regulators [83]. Interestingly, phosphorylation of MR is also implied as a cell type-specific mechanism, modulating MR activity. Hyperkalaemia was found to increase MR phosphorylation at S843 and subsequently prevent ligand-binding and receptor activation, a phosphorylation site specific in renal intercalated cells [84]. Intriguingly, the phosphorylation of MR can also regulate the ubiquitylation state and the subsequent degradation of the protein [85].

The epigenetic writer proteins HDACs are generally perceived as corepressors of nuclear receptors [86]. The attenuating effects on gene transcription of the earliest described corepressors, nuclear receptor corepressor (NCoR) and silencing

mediator of retinoid and thyroid receptor corepressor (SMRT) rely on the subsequent recruitment of HDACs [72]. Different studies could reveal that increased acetylation of MR, induced by HDAC inhibition, interferes with MR recruitment onto the DNA and attenuates transactivation of MR target genes [87, 88]. HDAC3 is acting as a coactivator in this context, as deacetylation of MR in the hinge region restores transcriptional activities of the receptor [88]. The treatment with HDAC inhibitors has also been linked to reduced antifibrotic effects in DOCA-salt-induced hypertensive rats and decreased expression of inflammatory markers in spontaneously hypertensive rats [17, 88, 89].

8. Summary

In summary, the distinct biological effects of MR in different cardiovascular cells are associated with changes in gene expression. Epigenetic modifications and modifying enzymes have been identified as crucial regulators of gene expression and cellular function in the cardiovascular system; however, presently available data on MR-dependent gene expression in the cardiovascular system is predominantly derived from experiments on cultured cells, in some cases after artificial overexpression of the MR, or tissue analysis. Analyses of complex tissues consisting of a dynamically changing mixture of multiple cell types can lead to ambiguous results. To date there is no ChIP-seq data published describing genome-wide MR-DNA interactions in cardiovascular cells. Studies in renal epithelial cells or brain tissue indicate that MR-DNA interaction can occur in promoter regions as well as at distal enhancer sites, but it remains speculative whether the insights from renal epithelial cells can be applied to the cardiovascular system as well.

This implies the necessity to perform cell type-specific studies from primary cardiovascular cells in homeostasis and different states of disease in order to identify distinct changes in gene expression and (epigenetic) mechanisms regulating MR activity in a given cell type (**Figure 2**). Utilisation of cell type-specific bulk or single-cell RNA-sequencing as well as integrated analysis of locus-specific histone modifications and DNA methylation, spatial organisation of the chromatin, MR-DNA binding and MR post-translational modification would allow a comprehensive insight into regulation of transcription by MR in cardiovascular cells and might lead to novel concepts for selective MR-targeting therapeutics.

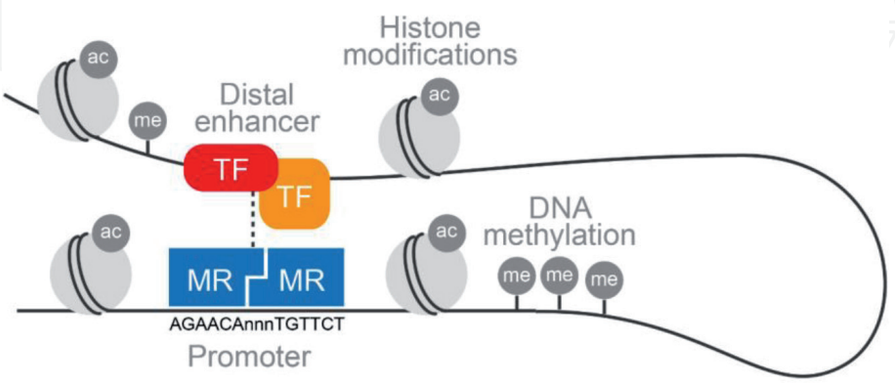

Figure 2.
Proposed epigenetic determents of MR transcriptional activity. Transcriptional activity of the mineralocorticoid receptor is determined by DNA methylation and histone modifications governing chromatin accessibility and activation status of promoter and distal enhancer regions. Transactivation of MR-bound promoter or enhancer regions involves spatial chromatin organisation and other transcription factors. MR, mineralocorticoid receptor; TF, transcription factor; ac, acetylation; me, methylation.

Acknowledgements

This publication is based upon work from the EU COST Action ADMIRE BM1301 in Aldosterone and Mineralocorticoid Receptor (MR) Physiology and Pathophysiology www.admirecosteu.com and was supported by the Else-Kröner-Fresenius-Stiftung (2016_ A163 to A. Lother).

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