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Nuclear Receptors in Vascular Biology

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Abstract

Nuclear receptors sense a diverse group of steroids and hormones (estrogens, progesterone, androgens, glucocorticoid and mineralocorticoid), vitamins (A and D), lipid metabolites, carbohydrates and xenobiotics. In response to these diverse but critically important mediators, nuclear receptors regulate the homeostatic control of lipids, carbohydrate, cholesterol and xenobiotic drug metabolism, inflammation, cell differentiation and development, including vascular development. The nuclear receptor family is one of the most important groups of signaling molecules in the body, and as such represent some of the most important established and emerging clinical and therapeutic targets. This review will highlight some of the emerging trends in nuclear receptor biology related to vascular biology.

Introduction

In man, the nuclear receptor family consists of 48 ligand activated-transcription factors [1]. For the purpose of this review I have subdivided these in to 5 functional groups: 1) the hormone/ steroid and related family, 2) the fat soluble vitamin and related family, 3) the nutritional and related family, 4) the xenobiotic sensing receptors and 5) the orphan / atypical receptors (Table 1). The majority of nuclear receptors have only recently been identified and remain orphan i.e. with no known endogenous ligand(s) [1]. Since, established sex, steroid, and vitamin nuclear receptors play such critical roles and are important clinical targets, there has been a great interest in elucidating the expression and function of orphan nuclear receptors. Our understanding of the great importance of nuclear receptors was initially based on only a few of these receptors (e.g. the steroid family) which have well-established ligands/ activators and roles throughout the body.

The vasculature is the interface between the circulation and underlying tissue, and as such is exposed to all the known nuclear receptor activators. Endothelial cells play a key role in cardiovascular homeostasis but are integral to all organ function. The endothelium has clear roles in blood pressure, regulating thrombosis, inflammation, transport and oxygen supply. A potentially important point of regulation of the vasculature could therefore be via nuclear receptors which are activated by circulating hormones, steroids, xenobiotics, and vitamins as well as other dietary nutrients including lipids and carbohydrates [1, 2]. Since the mid 1999's there has been a great interest in nuclear receptors in vascular biology. From a relatively small level of understanding that a number of steroid family members were expressed (estrogen [ER], glucocorticoid [GR], androgen [AR], mineralocorticoid [MR] and retinoic acid receptor [RAR]s) a functional role for a great number of vascular nuclear receptors including peroxisome proliferator activated receptors (PPAR-α,– β/δ $\alpha\nu\delta$ –γ), farnesoid X receptor (FXR), the small heterodimer partner (SHP), the pregnane X receptor (PXR), retinoid X receptors RXRα, –β and -γ, the constitutive androsterone receptor (CAR),

neuronal growth factor-induced clone B (NGFI-B)s, retinoic acid receptor-related orphan receptor (ROR)s, estrogen-related receptor (ERR)s and chicken ovalbumin upstream promoter-transcription factor (COUP-TF)s nuclear receptors have all been found expressed in vascular cells or tissue (Table 1, Table 2 and Figure 1A; [1]).

With the emergence of large scale omic strategies (e.g. gene array) signals for a large number of nuclear receptors (EAR-2 (COUP-TFγ), COUP-TFα, liver X receptor (LXR)β, ERRα, testicular orphan receptors (TR)2, RORα, TR4, COUP-TFβ, AR, liver receptor homologue (LRH)-1, RARβ, MR, GR, thyroid receptor (TR)α, REV-ERBβ, RORα, steroidogenic factor (SF)-1, and TRβ have been identified (table 2; figure 1; [2]). These data highlight that there is still very limited information (SHP, REV-ERBβ, COUP-TF, TR, ThR, VDR) or no information (ERR, GCNF, TR, HNF4) for vascular roles for many of these nuclear receptors. It must also me noted that these gene array studies, although useful, are not definitive and have several limitations, in that i) studies from tissue such as whole intact heart and aorta are unlikely to reveal any clear information regarding expression of nuclear receptors in a single rarer cell populations e.g. endothelial cells; ii) steady-state mRNA levels of any nuclear receptor does not necessarily correlate well to steady-state protein levels or importantly receptor activity; and iii) receptor levels may change upon cell activation. For three of the lowest level receptors indicated, PPARβ/δ, FXR, PXR (table 2) they already have a confirmed vascular cell expression [3-6].

This review aims to introduce the nuclear receptor family as an important family of therapeutically relevant signaling molecules and discuss the recent trends in their research in vascular biology, in particular to relation to cardiovascular diseases.

Nuclear receptor mode of action

Nuclear receptors are grouped as a family due to their structural similarity. The template for the nuclear receptor is a variable n-terminal AF-1 region, a relatively well conserved DNA binding domain,

a small hinge region, a ligand binding domain, and a c-terminal AF-2 domain which is often required for ligand dependent receptor activation [7]. There are several molecular mechanisms by which nuclear receptors are believed to act, however very little is still known about the potential for vascular specific actions. Nuclear receptors act in large complexes to induce transcription, trans-suppression and act vi non-genomic mechanisms:

Transcription: Nuclear receptors act in large dynamic complexes, commonly as homodimers or heterodimers (commonly with RXRs). Upon agonist treatment, nuclear receptors from a repressed protein complex undergo a conformational change and recruit a variety of activator proteins that are critical to initiate gene transcription i.e. genomic signalling [8]. Classically this co-activator complex involves the binding of the 'AF-2' c-terminal end of the ligand binding domain of the nuclear receptor to a LXXLL peptide sequence in the nuclear receptor co-activator [8]. The specificity of these nuclear receptor dimers in inducing target gene transcription is considered to be due to subtle differences in 'response elements' binding sites in target gene promoters. Nuclear receptors have a response elements based on repeats (e.g. direct or inverted), separated by different lengths of nucleotides of the sequence "AGGTCA".

Trans-suppression: Nuclear receptors can also trans-suppress gene activation independent of transcription e.g. the glucocorticoid receptor can directly bind and inhibit NFκB. Recently, a particular post-translational modification of nuclear receptors, SUMOylation, was identified as critical for many trans-suppression pathways. Ligand dependent SUMOylation of PPARγ or LXR mediates the anti-inflammatory effects of these receptors by physically stabilizing pro-inflammatory transcription factors in a co-repressor complex [9], while SUMOylation of LRH-1 in the liver can lead to increased atherosclerosis in atheroprone mice due repression of reverse cholesterol transport [10].

Non-genomic: There is increasing evidence, including studies in anucleated platelets that nuclear receptors can act in a rapid DNA-independent manner. RXRα, RXRβ, PPARγ, PPARβ/δ and the

glucocorticoid receptor are present and rapidly inhibit platelet activation [11-14]. RXRα was found to rapidly bind and inhibit the heterotrimeric G-protein Gq in a ligand dependent manner, potentially through the presence of an LXXLL motif found in the Gq family [14].

Nuclear receptors have great structural similarity particularly those of close family members, how they act to induce distinct but sometimes overlapping gene programs and functional effects is however far from clear. The role of co-regulators is clearly important [15] and may give cell and target specificity for a nuclear receptor. Well over 100 different proteins are known to interact with nuclear receptors, as co-activators, co-suppressors, and molecular chaperones (see the Nuclear receptor signalling atlas; http://www.nursa.org/). Figure 1C highlights some of the most highly expressed in human endothelial cells. Although less is known about these nuclear receptor- co-regulator interactions in vascular cells, in macrophages the PPARγ coactivator (PGC)-1α was recently found to be a target for the anti-atherosclerotic actions of conjugated linoleic acid [16], while BLC6 release from a complex with PPARβ/δ appears responsible for many anti-inflammatory actions of PPARβ/δ ligands in this cell type [17]. An additional emerging area also is the potential for nuclear receptor interaction with microRNA (miRNA)s. miRNAs are a family of a small non-coding RNA molecules which can silence mRNAs and regulate gene expression in a post-transcriptional manner [18]. Again recent data from macrophages indicate that activation of PPARγ inhibits the expression of miR-613 which in turn alleviates a suppression on LXRα and ABCA1 to promote reverse cholesterol transport [19].

New trends for nuclear receptors in regulating vascular biology:

There has been a great interest in particular in nutritional receptors including PPARs LXRs, FXR and RAR/ RXRs . These receptors have been reviewed extensively elsewhere. Here therefore the focus will be on the re-emergence of 1) steroid hormones and related receptors, 2) the emergence of

vascular xenobiotic receptors as novel vascular targets, and 3) orphan/ atypical receptors.

Steroid hormones and related receptors

The evidence that pre-menopausal women are protected from cardiovascular disease has long implicated the balance of the sex hormones estrogens and androgens in cardiovascular health [20-22]. ERα, ERβ [20] and AR [23] are expressed in vascular tissue. New evidence supports both a protective role for estrogen in vascular tissue, but also highlights a detrimental role for androgen signaling in macrophages, but not necessarily vascular cells in atherosclerosis development.

Proteases inhibitor use such as ritonavir commonly used to treat HIV is commonly associated with a profound dyslipidaemia [24]. Experimentally ritonavir increased coronary artery wall thickness and foam cell formation, which was associated with a down-regulation of ERα and Erβ, and reversed by addition of estradiol [25]. Moreover, ritonavir could be seen to directly bind ERα and inhibit its nuclear translocation (essential for its transcriptional activity) [25].

In contrast, Huang et al., have been studying the cell specific roles of androgens and the AR in atherosclerosis development [26]. Monocyte/macrophage specific AR knockout, endothelial cell specific-AR knockout, and smooth muscle cell specific -AR knockout mice were generated by cre-lox and then crossed with atheroprone LDLR knockout mice. Monocyte/macrophage specific AR knockout mice, or targetting AR degradation with a pharmacological agent ASC-J9 had reduced atherosclerosis compared with wild-type-LDLR knockout control mice, whereas endothelial cell and smooth muscle cell AR knockout mice were similar to control LDLR knockout mice[26].

The role of mineralocorticoid activation of MR to regulate aldosterone and blood pressure are well established. Moreover, MR inhibitors clinically reduced the incidence of cardiovascular events and improve mortality rates [27]. MR is also expressed in vascular endothelial cells and smooth muscle cells [27]. Smooth muscle cell MR activation causes vasoconstriction and promotes

vessel inflammation, fibrosis, and remodeling [27]. In a model of high fat feeding induced endothelial cell dysfunction, endothelial cell expression of NADPH oxidase (subunits p22phox, p40phox), and impaired endothelial cell-dependent vasodilation were reversed by either an MR antagonist (eplerenone), or in endothelial cells specific MR knockout mice [28].

Steroid receptors clearly have dramatic whole body actions including on metabolic pathways. These studies are also highlighting their expression in vascular tissue and are pointing to roles of these receptors directly in cell types relevant to the development of atherosclerosis.

Xenobiotic receptors

PXR is a nuclear receptor that has a highly promiscuous ligand binding domain, acting as a common drug, hormone, and nutrient sensor that co-ordinates detoxification and elimination pathways in the liver [29]. Although a number of PXR-regulated enzymes such as CYP3A and 2C are among the numerous CYPs expressed in vascular cells [30, 31], virtually nothing was known about the expression or relevance of PXR in vascular tissue. Recently published [5] results show that human primary vascular endothelial and smooth muscle cells express PXR and respond to PXR activation resulting in a cyto-protective, repair and anti-inflammatory phenotype; raising the possibility that vascular homeostasis in humans is directly influenced by this receptor. Given that the vasculature is an organ system estimated to have a mass equivalent to that of the liver [32] vascular PXR could play a pivotal role in whole body as well as in local vascular protection. In addition to vascular cells, PXR is also known to be expressed in human monocytes and T-lymphocytes [33]. Recent studies show PXR activation limits pro-inflammatory responses in monocytes and CD4+ T-cells (using cells isolated from the humanised PXR mouse; [34])

PXR in its role as a metabolic sensor, can be activated and regulate the activity of an array of drugs with high relevance to the cardiovascular system. PXR is activated by synthetic glucocorticoids;

dihydropyridine calcium channel blockers [35], statins [36], environmental contaminants (e.g. polychlorinated biphenyls; [36]), and cholesterol metabolites (e.g. oxysterols); all of which that have been implicated in atherosclerosis development [37, 38]. In addition, PXR promotes the CYP3A4-dependent biotransformation of clopidogrel to its active metabolite, thus promoting its inhibitory action on platelet aggregation [39]. Through PXR activation vascular tissue was able to convert the inactive pro-drug clopidogrel to its active metabolite [5]. A number of these findings were recently confirmed *in vitro* and *in vivo* [40] and extended to show that PXR activity is up-regulated in regions of laminar shear stress (athero-protected) but depressed with oscillatory shear stress (pro-atherogenic) in the mouse [40].

Despite the demonstration that PXR has protective roles in human vascular cells [5], studies in mouse models (particularly the mouse knockout) are far more inconsistent. PXR-apoE double knockout mice have a reduced atherosclerotic burden [41, 42] and PXR-ob/ob mice or PXR knockouts on a high-fat diet show increased obesity and insulin resistance [43]. A strong note of caution must however be made when interpreting the PXR knockout mouse, as distinct functional differences are found between murine and human PXR in the humanized-PXR mouse and in animal models where pharmacological drug responses have been observed. Humanized-PXR mice are resistant to high-fat diet-induced obesity, similar to that found in PXR knockout mice [44], findings that are consistent with a protective function for human PXR and that question the value of interpreting the previous knockout mouse studies as relevant to humans. In further support of a protective role for PXR, pharmacological activation using St John's Wort extract (containing the potent PXR activator hyperforin) improved lipid profiles and reduced atherosclerosis in the high-fat fed rabbit [45]. Similarly, pharmacological PXR activation in obesity prone AKR/J mice prevents high-fat diet-induced obesity and insulin resistance [46]. In contrast, Bisphenol A appears to increase atherosclerotic burden in the humanized PXR-apo knockout mouse (Bisphenol A increases atherosclerosis in

pregnane X receptor-humanized ApoE deficient mice [47]. Which if any of these in vivo studies truly represent the actions of PXR in humans with atherosclerosis is therefore far from clear. It is also conceivable that different activators may have different actions in different and the same tissue using the same nuclear receptor. What has emerged however is that the vasculature is a dynamic site for drug metabolism pathways via PXR.

Orphan/ atypical nuclear receptors.

Although much less is known about the orphan / atypical nuclear receptors, this is one of the most exciting areas of research. For example, COUP-TF family members, in particular COUP-TFII, is critical for vascular development [48]. COUP-TFII is expressed in the tissues in all major physiological systems: central nervous system (CNS), endocrine, metabolic, gastrointestinal, immune, reproductive, cardiovascular, respiratory and structural; with particularly high levels in the adrenal gland, kidney, ovary, uterus and vas deferens. COUP-TFII is vital for organogenesis, particularly of the vascular system [48, 49]. In mouse models, targeted deletion of COUP-TFII produces non-viable embryos with the heart atria and sinus venosus unable to develop past the primitive tube stage [48]. In addition the re-modelling of the capillary plexus into large and small micro-capillaries is defective. Conditional knockouts of COUP-TF II have additionally revealed a role in widespread organogenesis including venous programming and vein identity [50], insulin secretion and sensitivity [51], stomach development [52], adipogenesis [51], male fertility [53], and limb and skeletal muscle development [54].

Of increasing recent interest has been the NGFI-B (NR4A) family of nuclear receptors [55]. This family of nuclear receptors has clear roles in immune cell selection and development [55]. NGFI-B (Nur77; NR4A1), NGFI-Bb (Nurr1; NR4A2), reduce whereas NOR-1 (NR4A3) exacerbates atherosclerotic vascular lesions in mice [55]. Targetting macrophage NGFI-B (Nur77) activation in

macrophages either by pharmacological activation or overexpression reduces atherosclerosis in apoE knockout mice [56]. NGFI-B/Nur77 also appears to be directly anti-inflammatory in vascular cells as *in vitro*, NGFI-B/Nur77 is expressed in human endothelial cells and when activated or overexpressed inhibits the production of the potent vasoconstrictor endothelin-1 via suppression of the AP-1 transcription factor [57].

Conclusion

The nuclear receptor family represents one of the most clinically important therapeutic targets e.g. glucorticoids, estrogens, progesterones etc. 48 nuclear receptors exist in man; however we still no relatively little about a number of these targets and their endogenous ligands. We do now know the vasculature contains a vast number of these receptors where they not only provide valuable information about the role of the vasculature in physiology and homeostatic processes, but have provided avenues of research for new drugs. Over the last 20 years a number of these receptors have been targeted with varying degrees of success for therapeutic treatment of cardiovascular diseases. Over the next 20 we are sure to see continuing advances in the field and increasing numbers of ways to target this important set of signaling molecules.

Table 1: The nuclear receptor subfamily sub-divided in to activators

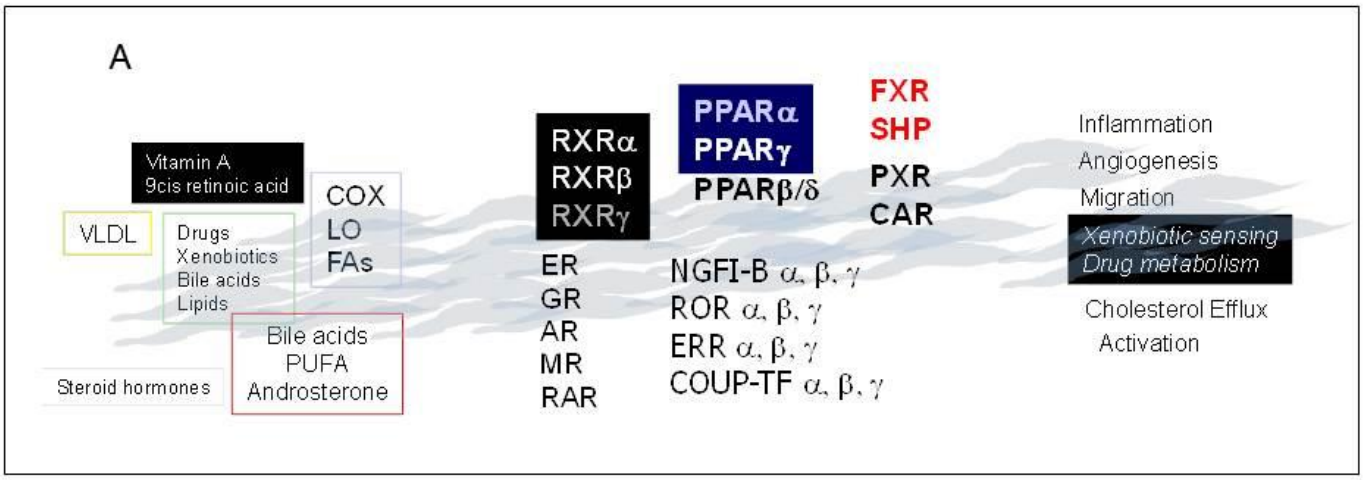
Steroid & related	Estrogen receptor α, -β, progesterone receptor, androgen receptor, glucocorticoid receptor, mineralocorticoid receptor, estrogen-related receptor –α, –β, –γ, steroidogenic factor-1, thyroid hormone receptor –α, –β
Vitamin & related	Retinoic acid receptor-α, –β, –γ, vitamin D receptor, retinoid X receptor-α, –β, –γ, retinoic acid receptor-related orphan receptor-α, –β, –γ
Nutritional & related	Peroxisome proliferator-activated receptor-α, –β, –γ, liver X receptor-α, –β, farnesoid X receptor, small heterodimer partner, hepatocyte nuclear factor 4, liver receptor homologue-1
Xenobiotic	pregnane X receptor, constitutive androsterone receptor
Orphan / atypical	chicken ovalbumin upstream promoter-transcription factor-I, -II, -II, germ cell nuclear factor, photoreceptor cell-specific nuclear receptor, RevErbAα, –β, tailless homologue, testicular receptor-2 and -4

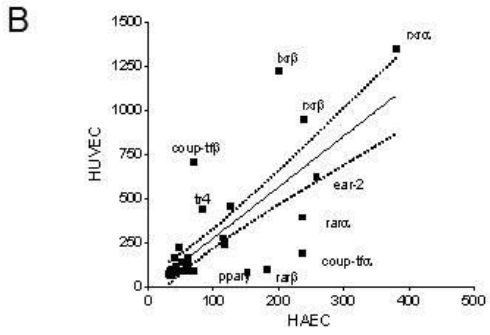
Table 2: Mouse cardiovascular (heart and aorta) nuclear receptor mRNA abundance (modified from ref [2]). Historical abbreviations have been used. [1]indicates receptors identified in human or rat vascular cells and tissue. Italics indicates an orphan receptor.

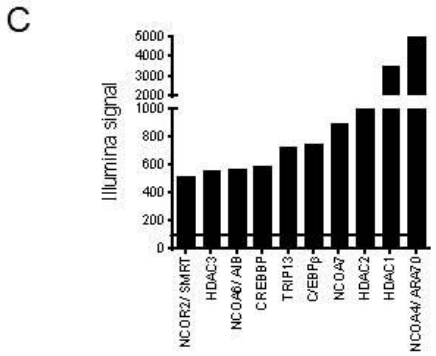
Absent	Low	Moderate				High
CAR[1]	AR	*RORβ*	*COUP-TFα*	MR	RARβ[1]	*COUP-TFβ*
DAX-1	ERβ[1]	*SHP*[1]	*COUP-TFγ*	*NOR1*	RARγ[1]	*ERRα*
FXRβ	FXRα[1]	*SF1*	ERα[1]	*NURR1*	*REV-ERBα*	LXRβ
HNF4α	*GCNF*	*TR2*	*ERRβ*	PPARα[1]	*REV-ERBβ*	*NGFIB*
PNR	*HNF4γ*	TR	*ERRγ*	PPARδ[1]	*RORα*	*RORγ*
PXR[1]	*LRH-1*	VDR	GR	PR	RXRα[1]	RXRβ[1]
TLX	PPARγ[1]		LXRα	RARα	*TR4*	RXRγ[1]
						TRα

AR, androgen receptor**, CAR**, constitutive androstane receptor, **COUP-TFα,–β–,γ**, chicken ovalbumin upstream promoter-transcription factor I, **Dax-1**, dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene, **ERα, -β**, estrogen receptor **ERRα, –β, –γ**, estrogen-related receptor, **FXRα**, –**β**, farnesoid X receptor, **GCNF**, germ cell nuclear factor, **GR**, glucocorticoid receptor **HNF4**, hepatocyte nuclear factor 4, **LRH-1**, liver receptor homologue, **LXRα, –β**, liver X receptor, **MR**, mineralocorticoid receptor, **NGFI-Bα, –β, –γ**, neuronal growth factor-induced clone B, **PNR**, photoreceptor cell-specific nuclear receptor, **PPARα, –β/δ, –γ**, peroxisome proliferator activated receptors, **PR,** progesterone receptor, **PXR/SXR**, pregnane X receptor/ steroid and xenobiotic receptor, **RARα, –β, –γ**, retinoic acid receptor, **RevErbAα, –β**, **RORα, –β, –γ**, retinoic acid receptor-related Orphan Receptor, **RXRα, –β, –γ**, retinoid X receptors, **SF-1,** steroidogenic factor, **SHP**, small heterodimer partner, **ThR**, thyroid hormone receptor, **TLL**, TLX or tailless, **TR-2, -4**, testicular orphan receptors, **TRα, –β**, thyroid receptor, **VDR**, vitamin D receptor.

Figure 1. Nuclear receptors in the vasculature. (A) Schematic of known nuclear receptors (central cluster), their activators (left cluster) and physiological roles (right cluster). (B) the correlation (r^2=0.6; p<0.05) between raw Illumina signals for nuclear receptors in the primary human aortic endothelial cells and human umbilical vein endothelial cells; (C) Raw illumine signals for the top 10 'expressed' nuclear receptor co-regulators in human primary aortic endothelial cells; mean of n=4 for each cell type.







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