



Nuclear receptor subfamily 5 group A member 2 (NR5A2): role in health and diseases

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Abstract

Nuclear receptors are the regulatory molecules that mediate cellular signals as they interact with specific DNA sequences. NR5A2 is a member of NR5A subfamily having four members (Nr5a1–Nr5a4). NR5A2 shows involvement in diverse biological processes like reverse cholesterol transport, embryonic stem cell pluripotency, steroidogenesis, development and differentiation of embryo, and adult homeostasis. NR5A2 haploinsufficiency has been seen associated with chronic pancreatitis, pancreatic and gastrointestinal cancer. There is a close relationship between the progression of pancreatic cancer from chronic pancreatitis, NR5A2 serving a common link. NR5A2 activity is regulated by intracellular phospholipids, transcriptional coregulators and post-translational modifications. The specific ligand of NR5A2 is unknown hence called an orphan receptor, but specific phospholipids such as dilauroyl phosphatidylcholine and diundecanoyl phosphatidylcholine act as a ligand and they are established drug targets in various diseases. This review will focus on the NR5A2 structure, regulation of its activity, and role in biological processes and diseases. In future, need more emphasis on discovering small molecule agonists and antagonist, which act as a drug target for therapeutic applications.

Keywords Nuclear receptors · NR5A2 · Chronic pancreatitis · Pancreatic cancer and NR5A2 regulators

Abbreviations

NR	Nuclear receptors
HREs	Hormone response elements
MR	Mineralocorticoid receptors
ER	Estrogen receptors
PR	Progesterone receptors
AR	Androgen receptors
GR	Glucocorticoid receptors
PXR	Pregame X receptor
SXR	Steroid xenobiotic receptor
CAR	Constitutive androstane receptor
LXR _s	Liver X receptors
NR5A2	Nuclear receptor subfamily 5 group A member 2

PPAR _γ	Peroxisome proliferator-activated receptor gamma
FXR	Farnesol X receptor
DLPC	Dilauroyl phosphatidylcholine
DBD	DNA binding domain
AF-1	Activation function-1
LBD	Ligand binding domain
AF-2	Activation function-2
SF-1	Steroidogenic factor 1
LRH-1	Liver receptor homolog
DUPC	Diundecanoyl phosphatidylcholine
SRC	Steroid receptor coactivator
SHP	Small heterodimer partner
Oct4	Octamer-binding transcription factor 4
PGC-1 α	PPAR _γ co-activator 1 α
MBF-1	Multiprotein bridging factor
CREB	CAMP response element-binding protein
C/EBP δ	CCAAT/enhancer-binding protein δ
CBP	Co-activators CREB binding protein
CYP7A1	Cholesterol 7 α -hydroxylase
Cyp 19	Aromatase
DAX-1	Dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1

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PROX1	Prospero-related homeobox 1
NCOR1	Nuclear receptor corepressor-1
SMRT	Silencing mediator for retinol and thyroid receptor
CYP8B1	12 α -Hydroxylase
HDAC3	Histone deacetylase 3 complex
GPS2	G protein pathway suppressor 2
PMA	Phorbol 12-myristate 13-acetate
PKA	Protein kinase A
SIRT1	SHP-sirtuin 1
PML	Promyelocytic leukemia protein
T1DM	Type 1 diabetes mellitus
AFP	α 1-Fetoprotein
HNF	Hepatocyte nuclear factor
Sox2	SRY-Box transcription factor 2
ERR β	Estrogen related receptor, beta
iPS	Induced pluripotent stem cells
LH	Luteinizing hormone
FSH	Follicular stimulating hormone
StAR	Steroidogenic acute regulatory protein
HSD3B2	3 β -Hydroxy steroid dehydrogenase type II
CYP 17	Cytochrome P450 17 α -hydroxylase
ChREBP	Carbohydrate response element binding protein
ChoREs	Carbohydrate response elements
GCK	Glucokinase
RCT	Reverse cholesterol transport
HDLs	High density lipoproteins
CETP	Cholesterol-ester-transfer protein
SR-B1	Scavenger receptor class B type I
BSEP	Bile salt export pump
ASBT	Apical sodium dependent bile acid transporter
Ost α/β	Organic solute transporter alpha–beta
MRP-3	Multidrug resistance protein-3
ER	Endoplasmic reticulum
IL-1RA	Interleukin-1 receptor antagonist
UPR	Unfolded protein response
PIK-3	Polio-like kinase 3
CYP11A1	Steroidogenic cholesterol side chain cleavage enzyme P450Sc
CYP11B1	11 β -Hydroxylase
NF-KB	Nuclear factor kappa-light chain-enhancer of activated B cells
AP1	Activator protein-1
CP	Chronic pancreatitis
PTF-1	Pancreas transcription factor
GWAS	Genome wide association studies
PDX-1	Pancreatic and duodenal homeobox 1
SNPs	Single nucleotide polymorphisms
PDAC	Pancreatic ductal adenocarcinoma
PanIN	Pancreatic intraepithelial neoplasia

Introduction

NR are regulatory molecules which function as transcription factors in a ligand-dependent way. Most of the NRs have a ligand-dependent activity, but some of NR family member does not have ligand, so they show constitutive activity [1, 2]. NRs interact directly with specific and short DNA sequences called hormone response elements (HREs) to modulate cellular signals to nucleus thereby acting as a central point of interaction between gene regulation and endogenous and environmental stimuli [2, 3]. The superfamily of nuclear receptor in humans has 48 members which are further divided into 7 subfamilies “NR0–NR6” [1, 4]. Transcription factors of NR superfamily perform an important role in cell death, embryonic development, differentiation, and metabolism [3].

Based on function, NRs can be divided into 3 different classes:

- Endocrine receptors triggered by ligands which have high-affinity steroid NRs. The example includes mineralocorticoid (MR), estrogen (ER), progesterone (PR), androgen (AR) and glucocorticoid (GR) receptors;
- Ligands for adopted orphan NRs example includes receptors for bile acids [Farnesol X receptor (FXR)], fatty acids [Peroxisome proliferator-activated receptor gamma (PPARs)], xenobiotics [pregame X receptor/steroid xenobiotic receptor (PXR/SXR) and constitutive androstane receptor (CAR)];
- Orphan NRs which controls transcription independent of ligands example includes Nuclear Receptor Subfamily 5 group A member 2 (NR5A2) [5, 6].

NRs control the gene expression involved mainly in homeostasis, embryonic development, and reproduction, like the involvement of NR5A2 in preliminary biological processes governing early development and stem cell pluripotency. Other receptors like PPAR γ controls glucose and energy metabolism homeostasis; PPAR α , β/δ , and γ are involved in lipoprotein, triglyceride, and fatty acid metabolism; LXRs, FXR, and NR5A2 are involved in bile acid metabolism; PXR/SXR plays a crucial role in defence against xeno- and endobiotics. The activity of maximum NRs is controlled by various lipid-soluble molecules, which include nutrients, steroid hormones, endo/xenobiotics, and metabolites. Ligand binding to the nuclear receptor causes conformational change which causes detachment and recruitment of co-repressors and co-activators leading to downstream target gene repression and activation respectively [7, 8].

Ligands have been discovered for about half of the superfamily members and these ligands are established

drug targets in many maladies like cancer, inflammation, and metabolic disorders. This creates interest in the recognition of orphan nuclear receptor ligands because of their ability to be used as potential drug targets to treat human disease. Classical medicinal chemistry strategy can be exerted to NR5A2 as dilauroyl phosphatidylcholine (DLPC) is shown to regulate its activity in vivo and it has a therapeutic application for type 2 diabetes. This finding from Moore lab indicates the importance of investigating the establishment of synthetic ligands targeting NR5A2 as this can lead to the discovery of other methods for metabolic diseases treatment [9].

As NR5A2 performs diverse functions, its involvement in various diseases is obvious. This review will focus on the NR5A2 structure, regulation of its activity, and role in biological processes and diseases. NR5A2 expression might be useful for the treatment of these disease but this might affect other functions of NR5A2. Regulation of NR5A2 can be explored further to exploit this nuclear receptor for therapeutic uses and various small molecule agonists and antagonist have been discovered which might be of therapeutic value.

Structure of NR5A2 and its family

All NRs shares a general conserved modular structure (Fig. 1) that includes DNA binding domain (DBD) or C region which consists of two zinc fingers and is highly conserved; A/B domain is amino-terminal to the DBD and is immensely variable among members of NR5A2 superfamily as it has activation function-1 (AF-1) which is ligand-independent; DBD carboxy-terminal known as E region or ligand-binding domain (LBD) and it also possesses conserved activation function-2 (AF-2) ligand-dependent motif playing an important role in co-activation interactions. Hinge domain, known as D region connects C region/DBD with region E/LBD [1, 4].

NR5A subfamily members like steroidogenic factor 1 (SF-1) and NR5A2 show a disparity in binding with DNA as compared to other NRs as they bind to DNA as monomers with high affinity. A 30 amino acid stretch called Ftz-F1 box is available at the COOH terminal of DBD of NR5A2 which specifically targets 5' extension (YCA) of consensus binding

site of Ftz-F1. YCA AGG YCR (Y-pyrimidine and R-purine) is a consensus binding site of the Ftz-F1 box [4, 10, 11].

NR5A2, a member of the NR5A subgroup have four members (NR5A1–NR5A4) [10]. It is positioned on the chromosome 1q32.11 with 8 exons spanning more than 150 kb [4]. It encodes Liver receptor homolog-1 (LRH-1) protein [12]. NR5A subfamily along with NR5A2 interacts with identical DNA consensus sequences, and their LBD can bind to phospholipids [10]. These receptors are in the category of orphan receptors, but bacterial phospholipids are known to be present in both SF-1 and NR5A2 binding pockets. It is unknown that whether these phospholipids are capable of modulating the activity of receptor or they are present only as structural or fortuitous ligands [1].

In humans, alternative splicing leads to at least three isoforms of NR5A2. The largest isoform LRH-1v1 is the most abundant isoform, LRH-1 of NR5A2 are identical having similar DNA-binding and transactivation capacities except a deletion on A/B region which corresponds to exon 2 in NR5A2. hLRH-1v2 is the smallest isoform which has further deletion in D and E regions because of alternative splicing in exon 5, causing its failure to activate transcription [4].

NR5A2 distribution in tissues

NR5A2 gene encodes transcription factor NR5A2 or LRH-1 which plays a significant role in multiple physiological processes like normal physiology, homeostasis, embryonic development, lipid metabolism, anti-inflammatory activities, cancer development, regulation of steroidogenesis and progesterone synthesis [12, 13]. In adults, NR5A2 is distributed in endodermal tissues like intestine, liver, and pancreas, which classifies it as an enterohepatic NR. NR5A2 also shows its expression in pre-adipocyte, ovary, and at lower levels in the placenta. Its expression is species-specific in adrenal gland and testis [4].

Activation of NR5A2

NR5A2 structure helps to understand its constitutive activity. NR5A subfamily members are considered as receptors with constitutive activity suggesting that in the absence of

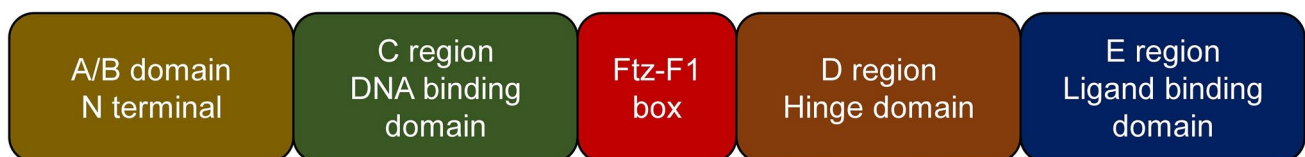


Fig. 1 Structure of NR5A2 encoded by NR5A2 gene

hormone binding, NR5A2 is transcriptionally active [7, 14]. The amino-terminal region of LBD consists of H1, H2, and H3, which are conserved and ligand-dependent [4, 10].

The 3-D structure of LBD of mouse NR5A2 (mNR5A2) consists of 12 α -helical conserved regions (H1-H12) folded into a triple-layer, helical antiparallel sandwich between H5 and H6 through a conserved β -turn constitute the LBD of most NRs. Different NRs have different size of ligand binding pocket of LBD. There is a limited sequence similarity of NR5A subgroup with other NRs subfamilies as N-terminal region of LBD and H1, H2 and, H3 of NR5A subgroup is conserved. The active site of NR is determined by H12, which consists of AF-2, which is stabilized by H2 [4]. AF-2 region folding permits active confirmation of LBD in the absence of ligand [3].

Unlike of mNR5A2, human NR5A2 (hNR5A2) binds to phospholipid ligands effectively because of marked structural features at the gateway of the ligand-binding pocket [15]. Thr439, Glu400 and Phe443 residues of H6–H7 in mNR5A2 reduce binding of phospholipid with mNR5A2 but in hNR5A2, homologous residues Ala, Gly and Leu assist binding of ligand on hNR5A2. Binding of ligand was ancestral in NR5A2 family but it diminished in mNR5A2 because of replacement of specific amino acids [16]. Hydrophobic ligand-binding pocket is present in both mNR5A2 and hNR5A2, which acclimatize C16:0, C18:0 acyl chains but an efficient lipid mimic of high affinity that can be held in this cavity is still evasive [15].

NR5A2 activity is modulated by diundecanoyl phosphatidylcholine (DUPC) and DLPC, which are direct ligands of the receptor. Their binding to NR5A2 and role in the recruitment of important co-activators like Steroid receptor coactivator-3 (SRC-3) is confirmed by mammalian two-hybrid assays [1]. Activation of NR5A2 might be independent of ligands even after possessing hydrophobic ligand-binding pocket as there is no effect on NR5A2 activity due to shape and size disruption of the cavity by bulky side chains. This

fact indicates that ligands for NR5A2 are inessential for basal activity but this does not deny the existence of NR5A2 ligands [17].

Nuclear receptors undergo a conformational change in ligand binding pocket upon binding of a ligand which results in stabilization of AF-2 surface of the co-activator binding interface of receptors resulting in the formation of charge clamp to aid in recruitment and binding of co-activators. But in contrast, the NR5A2 crystal structure demonstrates the role of the 4th sandwich layer created by helix 2 (H2) in the co-activator stabilizing interacting region of the LBD. When the ligand is not available, H2 is near H12 to constitutively stabilize AF-2 which aids in co-activator binding [10].

Regulation of NR5A2 activity

NR5A2 activity is constitutive, and its activation causes a conformational change which leads to co-repressors dissociation and incorporation of co-activators [18]. NR5A2 acts as a regulator of various pathways of metabolism, steroidogenesis, cancer, and pluripotency regulation. NR5A2 activity regulation might be helpful in the treatment of various diseases like gastrointestinal tract and pancreatic cancer [3]. NR5A2 functions are regulated (Fig. 2a) through:

- (1) Intracellular phospholipids
- (2) Transcriptional co-regulation: co-repressors and co-activators of NR5A2
- (3) Post-translational modifications
- (4) Synthetic ligands [3, 18]

Phospholipids as NR5A2 ligands

Various phospholipid derivatives like phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, as well as second messengers like phosphatidylinositol (PI: P1(3,5)P₂)

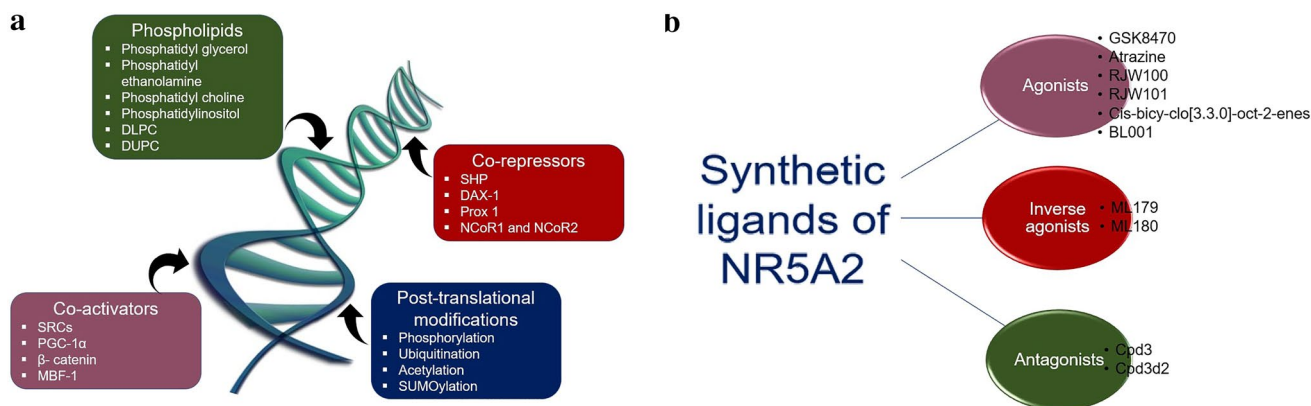


Fig. 2 a Regulatory mechanism of NR5A2. b Synthetic ligands of NR5A2

Table 1 Regulators of NR5A2

Co-activators of NR5A2	Activity of co-activators	References
1. SRC	a. Escalates hLRH-1 mediated activation of Cyp7A1 promoter in Huh 7 cells	[20]
2. PGC-1 α	a. Induces Cyp7A1 and Cyp9A1 expression in liver and breast respectively b. Activates NR5A2 in ovary leading to differentiation of granulosa cells into luteal cells	[21–23]
3. β -catenin	a. Acts synergistically with NR5A2 in intestinal crypt cells and promotes proliferation	[24]
Co-repressors of NR5A2	Activity of co-repressors	References
1. SHP	a. Blocks interaction of PGC-1 α and NR5A2 in hepatocytes and breast adipose stromal cells b. Represses activity of Cyp7A1 and Cyp8B1 c. Represses induction of carboxyl ester lipase promoter via NR5A2	[7, 21, 27, 29]
2. DAX-1	a. Blocks PGC-1 α and NR5A2 interaction	[23]
3. Prox 1	a. Suppresses Cyp7A1 transcription	[32]
4. NCoR1 and NCoR2	a. Represses acute phase response proteins via interaction of SUMOylated NR5A2 with NCoR1/HDAC3 complex	[19]

and PI(3,4,5)P₃) bind with NR5A2 ligand-binding pocket thereby indicating that phospholipid acts as endogenous ligands [7, 19]. Binding of specific phospholipids with NR5A2 is reduced by F343W, F416W mutations in LBD of NR5A2, which hampers co-activator recruitment and reduces the NR5A2 transcriptional potential [19]. RJW100 and DLPC bind to hNR5A2 but, neither of them fully fills the pocket nor mimics signalling phosphatidylinositol head groups which are exposed and assist in the stabilization of solvent-exposed residues present at the entrance of pocket [15]. DLPC and DUPC are two phospholipids identified as NR5A2 direct ligands, modulating the activity of receptor as DLPC and DUPC activate promoters specific for NR5A2. The examples include small heterodimer partner (SHP) and Octamer-binding transcription factor 4 (Oct4), and these phospholipids are specific for NR5A subfamily [1]. DLPC induce NR5A2 activity as shown during treatment of hepatocytes and also induce expression of those genes which are required for bile acid synthesis.

Though NR5A2 can be active without ligand binding, its transcriptional activity is further enhanced via binding of specific phospholipid [19].

NR5A2 regulators

Regulators of NR5A2 are co-activators of NR5A2 and co-repressors of NR5A2 (Table 1).

NR5A2 co-activators

NR5A2 has an entire functional AF-2 domain which contains hexameric LIIEML motif, essential for stabilization of the binding of co-activator. NR5A2 with truncated AF-2 is the dominant-negative protein [4]. NR5A2 is in an active conformation permanently, and as a monomer binds to DNA.

NR5A2 shows binding with transcriptional complexes and other orphan NRs, leading to an increase in transcription of target genes [3]. Binding of hepatic FXR with NR5A2 leads to stimulation of SHP, pyruvate carboxylase and retinol dehydrogenase 9 and phospholipid ethanolamine M-methyl transfer [3, 18]. Other co-activators which enhances NR5A2 activity are SRC, PPAR γ co-activator 1 α (PGC-1 α), β -catenin and Multiprotein bridging factor (MBF-1) [19].

SRCs SRC p160 family has three similar members, SRC-1, SRC-2, SRC-3 and they bind to LBD of NR5A2 and hence increases NR5A2 transcriptional activity [19]. The SRC homologs have LXYLL motif in their receptor interaction region and sequences of short peptide obtained from them binds to NR5A2 via LBD [3]. NR5A2 and cAMP response element-binding protein (CREB) interaction is increased by CCAAT/enhancer-binding protein δ (C/EBP δ), co-activators CREB binding protein (CBP) and SRC-3 [19]. In human adipose stromal cells, SRC-1 and SRC-3 increase NR5A2 dependent aromatase promoter transcription. AF-2 is required for interaction with transcriptional intermediary factor 2 and SRC-1 [18]. Overexpression of SRC-1 escalates hLRH-1 mediated activation of cholesterol 7 α -hydroxylase (Cyp7A1) promoter leading to an elevation in the transcription of Cyp7A1 in Huh 7 cells [20].

PGC-1 α PGC-1 α binds to AF-2 region of NR5A2 and induces Cyp7A1 and aromatase (Cyp9A1) expression in the liver and breast respectively [21, 22]. PGC-1 α also activates NR5A2 in the ovary, enabling granulosa cells differentiation into progesterone generating luteal cells. DAX-1 (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1) in granulosa cells blocks NR5A2 activity indicating the fact that DAX-1 has a high binding affinity than PGC-1 α [23].

β -catenin The synergy between β -catenin and NR5A2 in intestinal crypt cells induces cell cycle genes expression and promoting proliferation. β -catenin enhances the transcriptional effect of cyclin E1 expression directed by NR5A2. Contrary to this, NR5A2 acts as β -catenin co-activator in cyclin D transcription regulation through binding of armadillo repeat of β -catenin to NR5A2 LBD [24, 25].

MBF-1 In a dose-dependent manner, MBF-1 stimulates NR5A2 activity through interaction with TATA-binding proteins either separately, or via transcription factor IID complex recruitment as LXXLL motif is absent [3, 18].

Co-repressors of NR5A2

Binding of Co-repressors to LBD of NR5A2 directly through canonical LXXLL motifs inhibits the NR5A2 transcriptional activity [26]. Various co-repressors in different organs are SHP, DAX-1, Prospero-related homeobox 1 (Prox1), nuclear receptor corepressor-1 (NCoR1), silencing mediator for retinol and thyroid receptor (SMRT or NCoR2) [18, 19].

SHP SHP has an overlapping expression with NR5A2 [18]. SHP is devoid of typical DBD and binds to NR5A2 on the C-terminal AF-2 domain. This interaction of NR5A2 and SHP occurs through NR5A2 residues Arg 361 and Glu334 that forms a charge clamp [3, 19]. Binding of SHP to the CoR domain of NR5A2 competes out co-activators [27]. SHP expression is induced by NR5A2, resulting in a decrease of NR5A2 expression in a negative feedback loop [28, 29]. SHP follows direct and indirect methods to represses NR5A2 activity. SHP competes with co-activators and inhibits NR5A2 indirectly. SHP directly binds to NR5A2 leads to repression of NR5A2 transcriptional activity [27]. Interaction of PGC-1 α and NR5A2 is blocked by SHP in hepatocytes and breast adipose stromal cells, leading to repression of NR5A2 activity [21]. Carboxyl ester lipase promoter activity induction mediated by NR5A2 is repressed by SHP [7]. As bile acids levels increase in the liver, transcription of SHP is initiated due to activation of FXR. Increase in SHP stops NR5A2 transcriptional activity and leads to the decreased activity of NR5A2 target genes like Cyp7A1 and 12 α -hydroxylase (Cyp8B1) [27, 29].

DAX-1 DAX-1 shows its expression in embryonic stem cells, ovary and testis. Mouse DAX-1 with NR5A2 LBD crystallization shows that its N-terminus LXXLL related motifs of DAX-1 interacts with NR5A2 [3, 19]. As indicated by amino acid substitution, NR5A2 favourably commune with L(1+)XXLL-related motif-containing Ser, Tyr, Ser and Thr at positions -2, +2, +2 and +6 respectively [30]. For DAX-1 mediated repression, L-terminus end is required and DAX-1 binds as a dimer with AF-2 domain

in a claw-like fashion with NR5A2 that extends into the ligand-binding pocket. DAX-1 does not repress the NR5A2 activity in embryonic stem cells of mouse, conversely, Oct4 gene transcription is activated by DAX-1. This activation via DAX-1 occurs because of steroid receptor RNA activator interaction with DAX-1 [3]. This fact points out the probability of co-regulator, allowing tissue-specific NR5A2 function. DAX-1 can block the co-regulator PGC-1 α and NR5A2 interaction [23].

Prox1 In human cell lines and *Drosophila*, Prox1 shows its expression in the liver, heart, and hippocampus but binds to distinct NR5A2 regions. Interaction of Prox1 occurs with both LBD and DBD of NR5A2 in discrete cell lines or may require entire LBD in *Drosophila*, but Prox1 represses analogous metabolic pathways in two organisms [19]. In the liver, both NR5A2 and Prox1 show expression and control development of hepatic stem/progenitor cell [31]. Direct interaction of Prox1 and NR5A2 occurs in a dose-dependent manner which suppresses CYP7A1 transcription mediated by hLRH-1 and hence regulates bile acid synthesis in the liver [32]. Prox1 affects hepatic cholesterol regulators via transcription as Prox1 shows high expression in the liver, and not in the intestine [33].

NCoR1 and NCoR2 Transcriptional co-repressor complex of NCoR1/histone deacetylase 3 complex (HDAC3) interacts with SUMOylated NR5A2 via association with G protein pathway suppressor 2 (GPS2), and hence repression of acute-phase response proteins occurs [19]. SMRT or NCoR2 also represses NR5A2 transcriptional activity through an indirect mechanism. There is a functional correlation between SMRT and NR5A2 but these two do not interact directly, but uncharacterized coregulators might bridge the repression function of SMRT and NR5A2 [20].

Post-translational regulation of NR5A2

Various post-translational modifications include SUMOylation, phosphorylation, ubiquitination, and acetylation regulates NR5A2 [19]. The hinge region of NR5A2 consists of serine residue (S238 and S243) which are phosphorylated either by mitogen-activated protein kinase ERK1/2 or by phorbol 12-myristate 13-acetate (PMA), and hence leading to an increase in the activity of NR5A2 [3, 19]. Phosphorylation of human NR5A2 at Ser-469 by protein kinase A (PKA) initiates steroidogenesis dependent on NR5A2 in breast cell lines and decreases glucocorticoid production dependent on NR5A2 in an intestinal cell line. This different effect of PKA on different cell lines indicates that PKA affects the activity of NR5A2 [34]. Acetylated NR5A2 in basal state binds to SHP-sirtuin 1 transrepressive (SIRT1) complex [35]. The activity of NR5A2 is modulated through

small ubiquitin-related modifier (SUMO) conjugation machinery as NR5A2 act as a direct substrate. E-3 SUMO-ligases target NR5A2 for SUMOylation at distinct and conserved lysine residues at the hinge domain and decrease its transcriptional activity. NR5A2 SUMOylation leads to translocation of the transcription factor to promyelocytic leukemia protein (PML) containing nuclear bodies from the chromatin and its actively transcribed target genes. Reversion of SUMOylation leads to release of NR5A2 from PML nuclear bodies causing induction of target gene expression [36]. SUMOylated NR5A2 leads to stabilization of co-repressors of NR5A2 such as NCoR1/HDAC3 complex and Prox1, which decreases the target gene expression [19].

Synthetic ligands

The existence of conserved large hydrophobic pocket within LBD of NR5A2 indicates the chances of binding of synthetic ligands which can modulate the activity of NR5A2 for therapeutic aspects. Both synthetic agonists and antagonists are developed (Fig. 2b). GSK8470 is another small molecule agonist developed synthetically. The herbicide atrazine is also known to activate NR5A2. 24-exo (RJW100) is constantly active agonist of NR5A2 target gene expression in various cell lines. Another synthetic molecule is RJW101 which is selective for NR5A2, but its activity is moderate [37]. In HepG2 cells and human hepatocytes, the foremost discovered small-molecule agonist of NR5A2 is hydrophobic substituted cis-bicyclo [3.3.0]-oct-2-enes which increase the NR5A2 target SHP gene expression [18]. BL001 is a small agonist of NR5A2 which hampers the development of hyperglycemia and immune-dependent pancreas inflammation in type 1 diabetes mellitus (T1DM) in murine models. BL001 also hold back apoptosis of β cell in islets of type 2 diabetes patients and elevates insulin secretion and β cell mass [38]. NR5A2 inverse agonists, ML179 and ML180 discovered by Busby et al. shows an inhibitory effect on breast cancer. Cpd3 and Cpd3d2 are two synthetic compounds which inhibit the transcriptional activity of NR5A2 and reduce the expression of NR5A2 target gene like SHP, Cyclin E1 and inhibits human cancer cell lines proliferation [17, 39].

Role of NR5A2 in normal homeostasis

Expression of NR5A2 is high in the intestine, reproductive tissues, exocrine pancreas, liver as well as in pre-adipocytes [40]. In the early embryonic development stage, NR5A2 shows its involvement in the differentiation of liver, intestine, and pancreas, whereas, in adults, it

regulates steroidogenesis and homeostasis of cholesterol/bile acid [18]. Some of the essential functions of NR5A2 in normal homeostasis are explained in further subheadings of this review (Fig. 3a).

NR5A2 in early development

During the early development, NR5A2 shows remarkably high expression in pluripotent stem cells of the mouse [41]. Expression of NR5A2 changes from early phases of embryo development to later. In embryonic stem cells, all cells of morula stage [E2.5], trophectoderm, and blastocyst stage [E3.5] show high NR5A2 expression which becomes restricted to visceral endoderm at egg cylinder [E5.5] stage and at this stage ectodermal cells do not show NR5A2 expression. The expression of NR5A2 is still present in primitive mesoderm and endoderm during gastrulation [E.6–E7.5]. NR5A2 abundance occurs in endoderm originated tissues like liver, pancreas, intestine, testis, bone, and various regions of the brain during mid-gestation [E8–E15] [7]. At embryo day 6.5–7.5, NR5A2 deficient mice showed lethality with features of visceral endoderm dysfunction [3]. NR5A2 expression is persistent in foregut endoderm during morphogenesis of the pancreas and liver. During foregut differentiation, progressive NR5A2 expression takes place in the intestine, liver, and both exocrine and endocrine pancreas [4]. NR5A2 manifests its adult expression profile later during development at E17.5 which is distinguished by constant expression in liver, exocrine pancreas and stomach epithelium [42].

NR5A2 controls the expression of embryonic proteins which are necessary for liver development. Marker of liver differentiation and visceral endoderm is α 1-fetoprotein (AFP), and its early expression is under the regulation of NR5A2. AFP expression is all over during fetal liver growth followed by extinction in the perinatal period [7]. Effective NR5A2 binding elements have been found in various genes of hepatocyte nuclear factor (HNF) such as HNF-1 α , HNF-3 β , and HNF-4 α gene which encodes proteins necessary for early hepatic differentiation. NR5A2 is seen to bind with the promoter of these genes, directing transcription of hepatic nuclear factors [4, 7]. NR5A2 promoter has conserved GATA elements. In endodermal derivatives differentiation, GATA factors propose the tight alliance between GATA and NR5A2 signaling [4].

NR5A2 and stem cell pluripotency

Oct4 is an essential factor to maintain embryonic stem cell pluripotency [43]. Oct4 expression occurs in pre-gastrulation embryo, oocytes, and primordial germ cells. Lethality of an embryo at the blastocyst stage to extreme dysrhythmic differentiation process in vitro has been reported due to Oct4

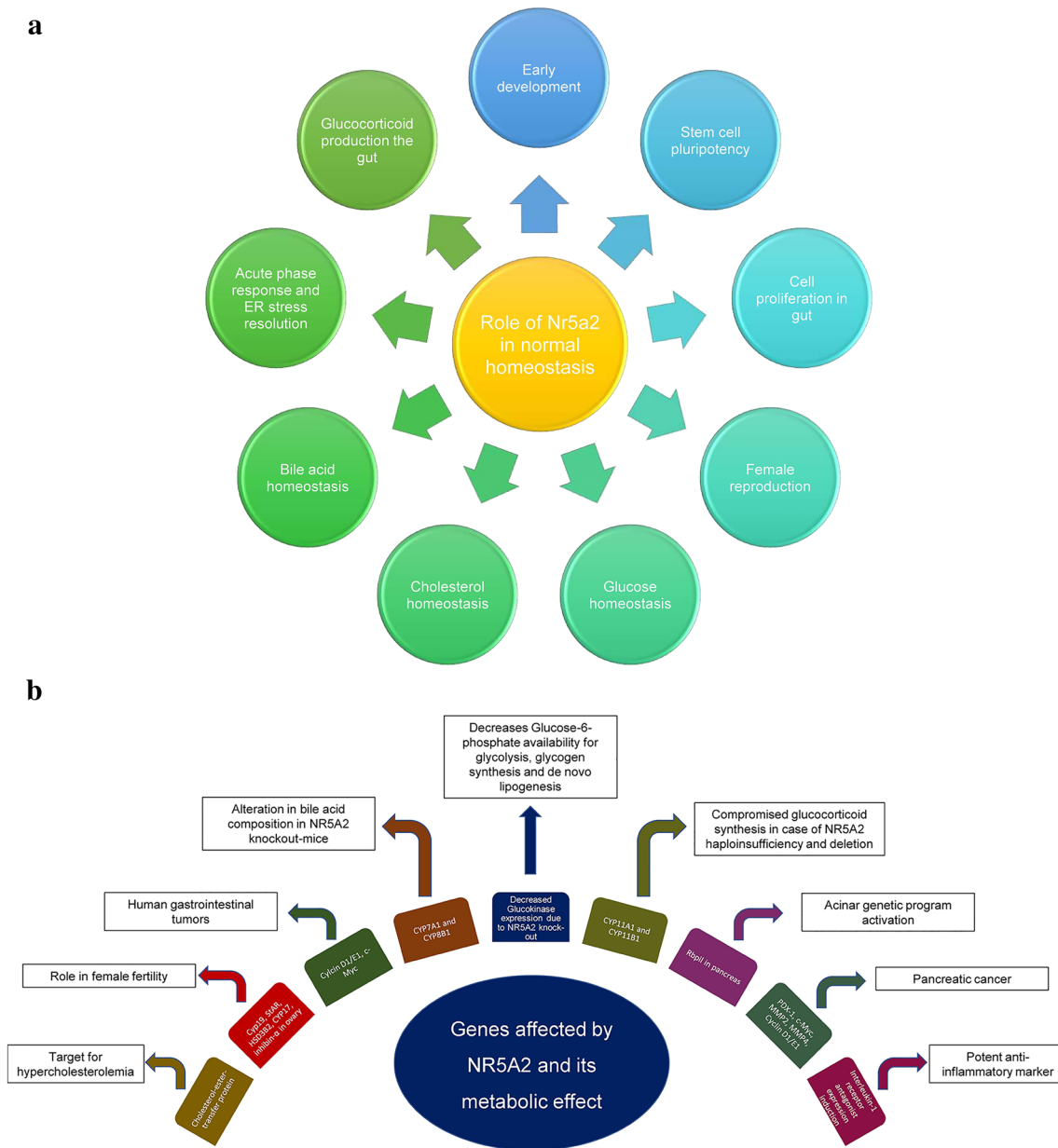


Fig. 3 **a** Role of NR5A2 in normal homeostasis. **b** Genes affected by NR5A2 and its metabolic effect

deficiency. Oct4 expression is induced at various stages of development by binding of many NRs to the Oct4 promoter. In the epiblast stage, NR5A2 seems to be pivotal for expression of Oct4 as loss of Oct4 expression has been observed in absence of NR5A2 [29].

Besides Oct4, NR5A2 together with SRY-Box Transcription Factor 2 (Sox2) and estrogen related-receptor beta (ERR β) is a demanding component of transcription factors regulatory network which maintains stem cell pluripotency [3, 7]. NR5A2 is crucial in Oct4 regulation and maintenance of pluripotency as to induce pluripotent stem cells (iPS) reprogramming in mouse embryonic fibroblasts, Oct4 can

be exchanged by NR5A2 [44]. Not only Oct4 but Nanog is also influenced by NR5A2 during embryonic stem cell pluripotency [43]. Induction of Nanog and Oct4 during early embryonic development mediated by NR5A2 is activated via the Wnt signaling pathway. In early embryonic stages, β -catenin binding turn on embryonic specific NR5A2 promoter and deficiency of β -catenin cause embryonic lethality [3, 7].

In embryonic stem cells, DAX-1 is in abundance, which acts as NR5A2 transcriptional partner to activate Oct4. NR5A2 and Nanog control DAX-1 expression [45]. Reprogramming capacity of NR5A2 is not affected by point

mutation within LBD of NR5A2, indicating binding of phospholipid is not necessary for this function [3].

NR5A2 and cell proliferation in the gut

The self-renewal capacity of epithelium is derived by pluripotent stem cells of the proliferative crypt compartment along with their uncommitted progenitors. Total epithelium renewal takes place every 4–5 days [46]. Progenitor cells come from dividing cells and differentiation of progenitor cells into specialized intestinal cell types like enterocytes; enteroendocrine cells are regulated by cell fate transcription factors expression like Notch/RBP-J/Hes, Gfi1, Math1, Mtgr1 and Hlf4 [47]. Intestinal epithelial layer integrity is regulated by NR5A2 as it speeds up damaged epithelium recovery by increasing stem and progenitor cell proliferation in intestinal crypts [48]. NR5A2 promotes the proliferation of cells by raising cell cycle cyclin mediated transition from G1 to S as shown by NR5A2 expression by retroviral transduction [7]. Accelerated cell cycle progression occurs via DNA dependent and independent transcriptional events as both are involved in the induction of the G1 cyclins. Hence, cell cycle progression is promoted by NR5A2 by two different and complementary mechanisms [41]. The first mechanism works in DNA binding independent manner in which transcription of c-Myc and cyclin D1 is induced by β -catenin/Tcf and co-activator of β -catenin is NR5A2. The second mechanism entails direct binding of NR5A2 to conserved response element of NR5A2 on cyclin E1 promoter, activated by β -catenin [7, 41]. NR5A2 doesn't have solely role in cell cycle regulation but it is also a co-activator as it interacts with β -catenin as simple DNA-binding transcription factor [7].

Inhibition of NR5A2 activity by cell-cycle inhibitors in epithelial cells of intestine indicates a connecting link of NR5A2 transcriptional activity and cell-cycle progression [48]. The primary signaling pathway stimulating proliferation and coordinating epithelial cells proliferation and differentiation transition along the crypt/villus axis is the canonical Wnt pathway. In this pathway, Wnt target gene expression is mainly initiated by the β -catenin/Tcf transcriptional complex [7].

NR5A2 in female reproduction

NR5A2 is one of the important regulators of ovary development and function [5]. NR5A2 expression is high in the ovary but confined to follicular granulosa cells and corpus luteum [3, 4]. No NR5A2 expression is seen in theca cells [3]. NR5A2 also plays a major function in the development of ovary, as specific ovarian patterns of its expression have been detected. Ovarian cholesterol intake for steroidogenesis can be controlled by NR5A2 [5]. During the estrous

cycle, expression of NR5A2 in the ovary is regulated by oestradiol and luteinizing hormone (LH) during ovulation, follicular growth by follicular stimulating hormone (FSH), luteinization and maturation of corpus luteum by LH and prolactin [26]. Progesterone production is regulated mainly over estrogen production by NR5A2 [49]. In luteal cells and granulosa, folliculogenesis, and ovulation is regulated by NR5A2 gene expression [26]. NR5A2 target genes within ovary are Cyp 19, steroidogenic acute regulatory protein (StAR), 3 β -hydroxy steroid dehydrogenase type II (HSD3B2), cytochrome P450 17 α -hydroxylase (CYP17) and inhibin- α [5, 26]. Transcription of these ovarian target genes in cultured cells is activated by NR5A2 binding to its response element in the promoter of these genes, indicating a key role of NR5A2 in affecting female fertility [26]. Anovulation is exhibited by granulosa cell-specific NR5A2 null mice because of cumulus expansion, luteinization and follicular rupture failure [50].

Glucose homeostasis by NR5A2

Extra- and intra-hepatic factors cooperate glycemic control. Expression of genes which are sensitive to glucose is directed by carbohydrate response element-binding protein (ChREBP) transcription factor which recognize gene promoter rich in conserved carbohydrate response elements (ChoREs) [51].

NR5A2 knockout mice under postprandial conditions show decreased glycogen synthase fluxes and glucokinase (GCK) because of decreased GCK expression as it is the NR5A2 target gene. This reduction in GCK expression causes low availability of glucose-6-phosphate which are the substrate for glycolysis, glycogen synthesis pathway and, de novo lipogenesis and also controls nuclear translocation and ChREBP activity [52]. As NR5A2 is an influential upstream regulator of the axis of GCK-ChREBP, NR5A2 activity might give an insight for the treatment of disease distinguished by irregular hepatic glucose sensing [51].

Systematic glucose homeostasis is also affected by glucose sensing in the liver which depends on NR5A2. Increased insulin secretion from the pancreas causes elevation in insulin levels and increase in the disposal of glucose because of compromised GCK mediated glucose consumption in liver-specific NR5A2 knockout mice [51]. Obese and diabetic mice having hepatic NR5A2 wild type on treatment with DLPC suggested an increase in insulin sensitivity, decrease in hepatic triglyceride levels, decrease in non-esterified fatty acids and less pale and fatty liver as compared to NR5A2 knockout mice treated with DLPC [1, 19].

Role of NR5A2 in cholesterol homeostasis

Cholesterol plays a key role in various cellular functions like biogenesis of membrane and steroid hormones biosynthesis. Elevation in cholesterol levels leads to multiple diseases such as, gallstones formation, cholestasis, and atherosclerosis. Regulatory circuit to control intracellular and circulating cholesterol levels includes reverse cholesterol transport (RCT), synthesis of bile acid and enterohepatic bile acid circulation, all of which are regulated by NR5A2 at some levels [4].

NR5A2 and RCT

RCT is a multistep process involving the transport of accumulated cholesterol from non-hepatic peripheral tissues mainly in the form of high-density lipoproteins (HDLs) to liver for excretion [4, 19]. Various RCT genes like *Scarb1*, *Abcg5* and *Abcg8* are transrepressed due to interaction of NR5A2 and PROX1, promoted by NR5A2 SUMOylation [33].

Cholesterol-ester-transfer protein (CETP) is one of the target genes of NR5A2, mainly distributed in small intestine and liver [4, 10]. CETP catalyzes the transfer of cholesteryl esters from HDL to lipoproteins rich in triglyceride followed by subsequent clearance of cholesterol by the liver, indirectly promoting RCT. Oxysterol activated NRs- LXR α and LXR β mediate an increase in CETP expression as a result of high cholesterol levels. NR5A2 strengthens this effect [53]. Various pharmaceutical companies have targeted CETP for hypercholesterolemia treatment [10].

NR5A2 regulates transcription of Apolipoprotein A1, molecule act as an acceptor for phospholipid and cholesterol from peripheral tissues leading to the formation of pre-HDL particles. On maturation of pre-HDL particles, scavenger receptor class B type I (SR-B1) transfers them to hepatocytes [10]. NR5A2 binding to the NR5A2 response element of the SR-B1 promoter induces SR-B1 expression. Haploinsufficiency of NR5A2 in mice leads to a decrease in SR-B1 expression, indicating its regulatory role in vivo [54].

NR5A2 in the homeostasis of bile acid

The prime way for cholesterol disposal from the body is the bile acid synthesis pathway as about 50% secretion of cholesterol is done by conversion to primary bile acids (cholic acid and chenodeoxycholic acid). Bile acid pool is maintained by either elimination through faeces or intestinal reabsorption followed by a return to the liver through enterohepatic circulation [4]. In bile acid biosynthesis, the rate-limiting enzymes are CYP7A1 and CYP8B1, essential for hydrophilic cholic acid production. CYP7A1 is transcriptionally

activated by NR5A2 [10]. NR5A2 heterozygous mice have been reported with elevation in CYP7A1 and CYP8B1 mRNA levels, indicating the dominant effect of NR5A2 on transcription of these genes [3]. In liver-specific NR5A2 knockout mice, alteration in bile acid composition due to decreased expression of CYP8B1 [55, 56].

Reabsorption of nearly 95% bile acids occurred in intestine and came back to the liver via the portal vein and this enterohepatic circulation has a dependence on various intestinal transporters which are regulated by NRs including NR5A2 [4]. Positive regulation of transcription of bile salt export pump (BSEP) and apical sodium-dependent bile acid transporter (ASBT) is mediated by NR5A2. NR5A2 also regulates basolateral bile acid export pumps like organic solute transporter alpha-beta (Ost α/β) and Multidrug resistance protein-3 (MRP-3). The expression of the hepatobiliary transport system is dramatically affected due to the loss of NR5A2 [40].

NR5A2 in acute phase response and endoplasmic reticulum (ER) stress resolution

Different inflammatory pathways have been linked with NRs and NR5A2. There are two aspects of inflammation response i.e. hepatic acute phase response and extra-adrenal glucocorticoid production in the gut, associated with NR5A2 [7]. The hepatic acute phase response is activated by cytokines secreted by immune cells into the bloodstream over inflammatory stimuli because of infection, injury, or chronic metabolic stress [19]. Group of proteins called acute-phase response proteins are secreted by hepatocytes [7]. Genes of acute phase response are transrepressed through SUMOylated NR5A2 binding to HDAC3 complex and NCOR1, mediated by GPS2. Thus, hepatic SUMOylated NR5A2 causes different co-repressor recruitment [19]. Expression of pro-inflammatory genes like haptoglobin, serum amyloid A and C reactive protein is reduced due to ectopic NR5A2 expression. NR5A2 also induces interleukin-1 receptor antagonist (IL-1RA) expression, which is a potent anti-inflammatory marker [10].

Independent of Unfolded protein response (UPR) canonical pathways, NR5A2 enhances ER stress resolution. Induction of stress response target genes occurs via phosphorylation of activating transcription factor by Polio-like kinase 3 (PIK-3). Defective ER stress resolution is displayed by liver specific LRH-1 knockout mice because of decreased PIK-3 expression. ER stress resolution can be rescued via PIK-3 ectopic restoration in liver specific LRH-1 knockout mice [57].

NR5A2 and extra-adrenal glucocorticoid production in gut

The adrenal gland is the main site for endogenous glucocorticoid production, serving in the regulation of metabolism and immune system [48]. Lungs and gut are involved in the synthesis and paracrine secretion of glucocorticoids for local immune cell homeostasis regulation [7, 48]. Transcription factors and mediators other than those in adrenals, seem to regulate local steroidogenesis. Functionally, SF-1 is substituted by its close homolog NR5A2 in intestine [48]. Injection of anti-CD3 antibody, which functions as a trigger for T cell activation in mice epithelial cells of intestine, induced counter-regulatory immune response. In the basal compartment of the mucosa of the intestine, this counter-regulatory immune response involves activation of steroidogenic cholesterol side-chain cleavage enzyme P450_{scc} (CYP11A1) and 11 β -hydroxylase (CYP11B1). In the intestine, this experiment points out the possibility of extra-adrenal glucocorticoid production as CYP11A1 catalyzes the conversion of cholesterol to pregnenolone and CYP11B1 converts deoxycorticosterone into corticosterone. Corticosterone interferes in pathways of proinflammation, initiated by nuclear factor kappa light chain- enhancer of activated B cells (NF- κ B) and Activator protein-1 (AP1) acting as inflammation inhibitor [58]. In the intestinal epithelium, targeted NR5A2 deletion and NR5A2 haploinsufficiency heavily compromise glucocorticoid synthesis mediated by immune cells [48]. Cell cycle inhibition in crypt-like epithelial cells diminishes NR5A2 activity and synthesis of corticosterone, indicating a close association between cell cycle and glucocorticoid synthesis [59].

NR5A2 in disease conditions

NR5A2 in gastrointestinal tumor and inflammation

Unconventional gene regulation in gastrointestinal tumors occurs because of altered signaling pathways and deregulated transcription factors. In 70–80% of colorectal cancer, Wnt/ β catenin signaling pathway is mutated. Cell proliferation is controlled for intestinal cell renewal, coordinated by NR5A2 via pathway of Wnt/ β catenin signaling. NR5A2 is involved in the formation of an intestinal tumor by way of its interaction with β -catenin/Tcf signaling by initiating cell proliferation and self-renewal mediated by cyclin D1/E1 specific of G1/S. In normal homeostasis, complex multi-protein interactions consists of GSK-3 β a serine/threonine kinase that maintains β -catenin levels by inducing deterioration of β -catenin. β -catenin destruction is prevented because of Wnt signaling activation leading to the association of β -catenin and co-activation of Tcf4 and further inducing

genes triggering cell cycle progression [18]. NR5A2 and β -catenin downstream targets are regulators of cell cycle i.e. c-Myc, cyclin E1, and cyclin D and overexpression of these genes shows association with human gastrointestinal tumors [41]. hLRH-1 and hLRH-1v1 are isoforms of NR5A2, which shows co-expression and significant up-regulation on human gastric cancer [18]. Effect of reduced NR5A2 expression in intestinal tumorigenesis was examined by Auwerx et al. using two different mouse models of disease. The first model of NR5A2 haploinsufficient APC^{min/-} mice had NR5A2 expression reduced and less tumor formation as compared to the expression of wild type NR5A2. In the second model, treatment of Azoxy methane, a chemical inducer of colon cancer in mice with one NR5A2 allele had significantly decreased abnormal crypt foci as compared to wild NR5A2 expression levels [10].

NR5A2 is the master regulator of the constitutive expression of steroidogenic enzymes in most tumor samples. As NR5A2 regulates both the cell cycle and glucocorticoid synthesis, NR5A2 might be regulated at two different levels in colon cancer i.e. tumor growth by regulation of proliferation and observe tumor development by immune cell suppression [48].

NR5A2 in chronic pancreatitis

In the pancreas, an inflammatory disorder caused pancreatitis and can be divided into acute and chronic pancreatitis (CP) [60, 61]. Inflammatory injury of the exocrine pancreas in pancreatitis seems to recover through the regeneration of acinar cells which produces digestive enzymes. Regeneration of acinar cells shows the involvement of brief inflammation phase, redifferentiation, and metaplasia derived by acinar cells, resident fibroblasts, and leukocytes. Any disparity between pro-inflammatory and pro-differentiation pathways leads to CP, which is identified by fibrosis and inflammation of acinar cells [62]. In CP, dysfunction of endocrine and exocrine gland develop irreversible structural damage to the structure of pancreas [61]. NR5A2 maintains normal homeostasis of the adult pancreas [63]. NR5A2 regulates pancreatic enzymes production as it is required for progenitor cell differentiation into acinar cells [62, 64]. NR5A2 directly regulates Rbpil, and it also coordinates with the pancreas transcription factor (PTF-1) complex in acinar genetic program activation. In adult mice, inactivation of NR5A2 causes decreased secretion of enzymes and pancreatic fluid [65]. In pancreatitis, NR5A2 activates the regeneration of functional acinar cells from malignant cells after inflammation which suggests that NR5A2 plays an important role in pancreas protection [66].

NR5A2, along with NOTCH1 and SMO promotes inflammation and metaplasia resolution in the pancreas, and the absence of any of these factors leads to persistent

inflammation and drives fibrosis through leukocyte stellate cell interactions [62]. Adult NR5A2[±] mice pancreata is histologically normal. But heterozygosity of NR5A2 increases chances of damage to the pancreas, impairs regeneration, and also shows cooperation with mutant KRAs in tumor progression [63]. Haploinsufficiency of NR5A2 in pancreatic acinar cells of mice showed an increase in the severity of pancreatic inflammatory response, high levels of pro-inflammatory cytokine mRNA levels, and activation of NF- κ B and STAT 3 pathways [65, 67]. Caerulein treated NR5A2 knockout mice shows phenotype similar to CP, indicating that reduction of NR5A2 activity imparts the pro-inflammatory phenotype of the metaplastic epithelium. NF- κ B induced metaplasia might target NR5A2 and PTF1-L, as inhibition of NR5A2 and PTF1-L may destabilize acinar cells [62].

The antagonist of hNR5A2, Cpd3 binds with LBD of NR5A2 and leaves NR5A2 transcriptionally inactive. Treating developing embryo of zebrafish after gastrulation with Cpd3 leads to distorted genesis of mature exocrine pancreas. In vivo inhibition of NR5A2 via Cpd3 leads to the conclusion that it can be used in the modification of differentiation and specification of hepatopancreas progenitors [39].

NR5A2 in pancreatic cancer

Prognosis of pancreatic cancer is worst among all the cancers [18]. In undifferentiated embryonic stem cells, NR5A2 is essential for the development of the pancreas and acinar feature maintenance in the exocrine gland. NR5A2 induces carboxyl ester lipase expression in the exocrine pancreas, which assists in cholesteryl ester absorption and lipoproteins assembly by the intestine [68]. In pancreatic cancer, genome-wide association studies (GWAS) showed a link between NR5A2 gene mutations and its upstream promoter region with pancreatic cancer [69]. In the pancreas, pancreas-specific transcription factors like Pancreatic and duodenal homeobox 1 (PDX-1) controls developmental pathways such as Wnt/ β -catenin, Hedgehog signaling, and regulatory cascades. NR5A2 is linked to these pathways controlled by PDX-1 and reactivation of these pathways occurs in pancreatic cancer, indicating their essential role in pancreatic cancer development [64]. High NR5A2 levels are found in cancerous cells nuclei and cytoplasm. Specific NR5A2 reduction strongly blocks the proliferation of cancer cells in multiple cell lines [14]. Phenotypic characteristics of pancreatic cell lines like wound healing, increased cell migration, invasion, and sphere formation can occur due to NR5A2 overexpression [18]. Activation of NR5A2 might not be the factor for PDX-1 reappearance, always indicating other regulatory mechanisms like genomic alterations might be involved in NR5A2 upregulation in pancreatic cells [64]. NR5A2 gene mapped 5 single nucleotide polymorphisms (SNPs), and its upstream regulatory regions have been found

associated with pancreatic cancer cells [63]. Among 5 highly significant SNPs (rs3790844, rs3790843, rs10919791, rs12029406, rs4465241) on chromosome 1q32.1 of NR5A2, rs3790844 reported positive association with the pancreatic ductal adenocarcinoma (PDAC) [69]. Many pancreatic cell lines show low levels of NR5A2 co-repressors, ProX and SHP, indicating the idea that NR5A2 transcriptional activity regulation changes may show association with pancreatic oncogenesis [64]. In some pancreatic cancer cells, considerable Nanog transcripts amplification is found. Nanog is a target gene of NR5A2, and its reappearance might be connected to dedifferentiated pancreatic cancer cell proliferation, associated with aggressive pancreatic tumors [7, 64]. NR5A2 involvement in pancreatic cancer stem cell maintenance is a key contributor to a pancreatic cancer relapse and its metastasis. NR5A2 blocking specifically through siRNA can inhibit proliferation of pancreatic cancer cell in vitro because of alteration in the transcriptional targets of NR5A2 which controls cell proliferation and differentiation [64].

In pancreatic cancer cell lines, NR5A2 overexpression causes an elevation in β -catenin transcriptional activation and downstream target genes such as c-Myc, MMP2, and MMP4 expression. In nude mice, NR5A2 upregulated cyclins D1/E1 and c-Myc genes hence promote pancreatic cancer cell or tumor proliferation [18]. Interestingly, in vitro proliferation can be inhibited through the blocking of NR5A2 activity in cell lines of pancreatic cancer as it leads to down-regulation of cyclin D1, E1 and c-Myc [39]. But role of NR5A2 in promoting pancreatic cancer is still controversial as some studies suggests that there is down-regulation of NR5A2 in acinar cells undertaking duct-like dedifferentiation which is necessary for acinar PDAC initiation. NR5A2 is required for acinar cell maintenance, not for their development. NR5A2 deletion causes destabilized mature acinar cells and worsen oncogenesis development driven by Kras [14]. Hence, there is a bifunctional nature of NR5A2 in the progression of PDAC: PDAC initiation is assisted by impaired function of protein through affecting the differentiation of acinar while overexpression at later stages causes PDAC growth advantage [70].

Association of chronic pancreatitis and pancreatic cancer

Pain, pancreatic insufficiency, metabolic borne disease and, PDAC are the end results of the last stage of CP [71]. It is thought that inflammation influences carcinogenesis via DNA damage and intracellular signaling pathways activation [72]. CP increases pancreatic cancer risk via a cascade of inflammatory processes that are supplemented by hereditary and somatic mutations [73]. One of the common features of CP and pancreatic cancer is fibrosis in which pancreatic

stellate cells switch to activated state from quiescent state in response to injury [62]. Pancreatitis and pancreatic cancer are linked by pancreatic stellate cells, resident pancreatic fibroblasts [62, 74]. Initiation of pancreatic cancer is also derived by acinar-cell differentiation loss indicating a link between CP and pancreatic cancer risk. Similar genes like *NR5A2*, *NOTCH1*, and *SMO* which are involved in assisting metaplasia and inflammation in pancreatitis, also in PDAC development. In humans and mice, the development of PDAC by CP is mediated partly by continuous inflammation-driven NF- κ B activation. Acinar cell-derived PDAC can be limited by *NOTCH1*, *MIST1*, and *NR5A2* [62]. In the mouse model, haploinsufficiency of *NR5A2* assists with pancreatitis directed by oncogene KRAs, enhancing pre-neoplastic pancreatic intraepithelial neoplasia (PanIN) lesions and development towards PDAC [66]. High risk of pancreatic cancer in patients with CP is affected by other multiple variables like shared risk factors for alcohol use, diabetes mellitus, and cigarette smoking. The high risk of PDAC in CP is supposed to be affected by chronic inflammation and over-proliferation of pancreatic stellate cells [71].

Hereditary pancreatitis is a rare and inherited form of CP resembling other forms of pancreatitis phenotypically except it appears in early age and strong family history [73, 74]. Inheritance of CP is autosomal dominant with point mutation on chromosome 7 [75]. Hereditary pancreatitis patients have a higher risk to develop pancreatic cancer, mainly due to extended duration of inflammation. Genetically determined idiopathic CP shows higher pancreatic cancer risk as compared to alcohol-related CP risk. Association of pancreatic cancer with hereditary pancreatitis as risk is about 70% time more than in the normal population. In contrast to other factors, less population attributable risk of pancreatic cancer is because of the low incidence and prevalence of CP [73].

Conclusion

Increased *NR5A2* activation might be useful in promoting biliary cholesterol excretion and RCT for the prevention of atherosclerosis development or in diabetes treatment, but on the other side, it can induce cell proliferation by inducing cell-cycle regulators expression and likely promote cancer development. Hence, *NR5A2* transcriptional programs could provide compelling therapeutic aspects.

NR5A2 expression is studied in various tumors like colon, gastric cancer, breast carcinomas, and pancreatic cancer. Therefore, exploring the possible influence of *NR5A2* modulators as a therapeutic target against various diseases should be considered. An example is the high expression of *NR5A2* in pancreatic cancer, and when blocked, downstream genes like cyclin D1, E1, and c-myc are down regulated, and the involvement of Kras in oncogenesis through

destabilization of mature acinar cells by *NR5A2*. Other examples can be given for diabetes, stem cell pluripotency, or female reproduction, etc. (Fig. 3b).

Sensitive *NR5A2* modulators can be developed through recent identification of phospholipid ligands and small molecule agonists. Since, *NR5A2* activity is intensively affected by coregulators, the prospect of modulation of *NR5A2*: coregulator interactions need to be examined besides looking for compounds that bind to cavity phospholipid/ligand-binding region. Hence, *NR5A2* ligand identification will not only discovers its verifiable biological roles and also open a new direction for novel treatment possibilities for a variety of diseases.

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References

1. Burris TP, Busby SA, Griffin PR (2012) Targeting orphan nuclear receptors for treatment of metabolic diseases and autoimmunity. *Chem Biol* 19:51–59. <https://doi.org/10.1016/j.chembiol.2011.12.011>
2. Zimmer V, Liebe R, Lammert F (2015) Nuclear receptor variants in liver disease. *Dig Dis* 33:415–419. <https://doi.org/10.1159/000371695>
3. Lazarus KA, Wijayakumara D, Chand AL, Simpson ER, Clyne CD (2012) Therapeutic potential of liver receptor homolog-1 modulators. *J Steroid Biochem Mol Biol* 130:138–146. <https://doi.org/10.1016/j.jsbmb.2011.12.017>
4. Fayard E, Auwerx J, Schoonjans K (2004) LRH-1: an orphan nuclear receptor involved in development, metabolism and steroidogenesis. *Trends Cell Biol* 14:250–260. <https://doi.org/10.1016/j.tcb.2004.03.008>
5. Mouzat K, Baron S, Marceau G, Caira F, Sapin V, Volle DH, Lumbruso S, Lobaccaro JM (2013) Emerging roles for LXRs and LRH-1 in female reproduction. *Mol Cell Endocrinol* 368:47–58. <https://doi.org/10.1016/j.mce.2012.06.009>
6. Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ (2001) Nuclear receptors and lipid physiology: opening the X-files. *Science* 294:1866–1870. <https://doi.org/10.1126/science.294.5548.1866>

7. Fernandez-Marcos PJ, Auwerx J, Schoonjans K (2011) Emerging actions of the nuclear receptor LRH-1 in the gut. *Biochim Biophys Acta* 1812:947–955. <https://doi.org/10.1016/j.bbadis.2010.12.010>
8. Francis GA, Fayard E, Picard F, Auwerx J (2003) Nuclear receptors and the control of metabolism. *Annu Rev Physiol* 65:261–311. <https://doi.org/10.1146/annurev.physiol.65.092101.142528>
9. Lee JM, Lee YK, Mamrosh JL, Busby SA, Griffin PR, Pathak MC, Ortlund EA, Moore DD (2011) A nuclear-receptor-dependent phosphatidylcholine pathway with antidiabetic effects. *Nature* 474:506–510. <https://doi.org/10.1038/nature10111>
10. Busby S, Nuhant P, Cameron M, Mercer BA, Hodder P, Roush WR, Griffin PR (2010) Discovery of inverse agonists for the liver receptor homologue-1 (LRH1; NR5A2). Probe Reports from the NIH Molecular Libraries Program, National Center for Biotechnology Information
11. Meinsohn MC, Smith OE, Bertolin K, Murphy BD (2019) The orphan nuclear receptors steroidogenic factor-1 and liver receptor homologue-1: structure, regulation, and essential roles in mammalian reproduction. *Physiol Rev* 99:1249–1279. <https://doi.org/10.1152/physrev.00019.2018>
12. Kaluarachchi DC, Momany AM, Busch TD, Gimenez LG, Saleme C, Cosentino V, Christensen K, Dagle JM, Ryckman KK, Murray JC (2016) Polymorphisms in NR5A2, gene encoding liver receptor homologue-1 are associated with preterm birth. *Pediatr Res* 79:776–780. <https://doi.org/10.1038/pr.2016.7>
13. Xu Z, Hou X, Lv H, Sun B, Cui Y, Liu L, Rong F (2017) Expression of liver receptor homologue-1 (LRH-1) in villi and decidua of patients with unexplained recurrent spontaneous abortion. *Med Sci Monit* 23:2445–2452. <https://doi.org/10.12659/msm.904645>
14. Fletterick R (2017) NR5A2 discovering compounds that block tumor growth in PDAC. *J Surg Oncol* 116:89–93. <https://doi.org/10.1002/jso.24639>
15. Miranda DA, Krause WC, Cazenave-Gassiot A, Suzawa M, Escusa H, Foo JC, Shihadih DS, Stahl A, Fitch M, Nyangau E, Hellerstein M, Wenk MR, Silver DL, Ingraham HA (2018) LRH-1 regulates hepatic lipid homeostasis and maintains arachidonoyl phospholipid pools critical for phospholipid diversity. *JCI Insight*. <https://doi.org/10.1172/jci.insight.96151>
16. Krylova IN, Sablin EP, Moore J, Xu RX, Waitt GM, MacKay JA, Juzumiene D, Bynum JM, Madauss K, Montana V, Lebedeva L, Suzawa M, Williams JD, Williams SP, Guy RK, Thornton JW, Fletterick RJ, Willson TM, Ingraham HA (2005) Structural analyses reveal phosphatidyl inositols as ligands for the NR5 orphan receptors SF-1 and LRH-1. *Cell* 120:343–355. <https://doi.org/10.1016/j.cell.2005.01.024>
17. Sablin EP, Krylova IN, Fletterick RJ, Ingraham HA (2003) Structural basis for ligand-independent activation of the orphan nuclear receptor LRH-1. *Mol Cell* 11:1575–1585. [https://doi.org/10.1016/s1097-2765\(03\)00236-3](https://doi.org/10.1016/s1097-2765(03)00236-3)
18. Nadolny C, Dong X (2015) Liver receptor homologue-1 (LRH-1): a potential therapeutic target for cancer. *Cancer Biol Ther* 16:997–1004. <https://doi.org/10.1080/15384047.2015.1045693>
19. Stein S, Schoonjans K (2015) Molecular basis for the regulation of the nuclear receptor LRH-1. *Curr Opin Cell Biol* 33:26–34. <https://doi.org/10.1016/j.ceb.2014.10.007>
20. Xu P-L, Liu Y-Q, Shan S-F, Kong Y-Y, Zhou Q, Li M, Ding J-P, Xie Y-H, Wang Y (2004) Molecular mechanism for the potentiation of the transcriptional activity of human liver receptor homologue 1 by steroid receptor coactivator-1. *Mol Endocrinol* 18:1887–1905. <https://doi.org/10.1210/me.2003-0334>
21. Shin D-J, Osborne TF (2008) Peroxisome proliferator-activated receptor- γ coactivator-1 α activation of CYP7A1 during food restriction and diabetes is still inhibited by small heterodimer partner. *J Biol Chem* 283:15089–15096. <https://doi.org/10.1074/jbc.M710452200>
22. Safi R, Kovacic A, Gaillard S, Murata Y, Simpson ER, McDonnell DP, Clyne CD (2005) Coactivation of liver receptor homologue-1 by peroxisome proliferator-activated receptor; coactivator-1A on aromatase promoter II and its inhibition by activated retinoid X receptor suggest a novel target for breast-specific antiestrogen therapy. *Can Res* 65(24):11762–11770. <https://doi.org/10.1158/0008-5472.CAN-05-2792>
23. Yazawa T, Inaoka Y, Okada R, Mizutani T, Yamazaki Y, Usami Y, Kuribayashi M, Orisaka M, Umezawa A, Miyamoto K (2010) PPAR- γ coactivator-1 α regulates progesterone production in ovarian granulosa cells with SF-1 and LRH-1. *Mol Endocrinol* 24:485–496. <https://doi.org/10.1210/me.2009-0352>
24. Yumoto F, Nguyen P, Sablin EP, Baxter JD, Webb P, Fletterick RJ (2012) Structural basis of coactivation of liver receptor homologue-1 by β -catenin. *Proc Natl Acad Sci USA* 109:143–148. <https://doi.org/10.1073/pnas.11170361108>
25. Botrugno OA, Fayard E, Annicotte J-S, Haby C, Brennan T, Wendling O, Tanaka T, Kodama T, Thomas W, Auwerx J (2004) Synergy between LRH-1 and β -catenin induces G1 cyclin-mediated cell proliferation. *Mol Cell* 15:499–509. <https://doi.org/10.1016/j.molcel.2004.07.009>
26. Zhao H, Li Z, Cooney AJ, Lan ZJ (2007) Orphan nuclear receptor function in the ovary. *Front Biosci* 12:3398–3405. <https://doi.org/10.2741/2321>
27. Lee Y-K, Moore DD (2002) Dual mechanisms for repression of the monomeric orphan receptor liver receptor homologous protein-1 by the orphan small heterodimer partner. *J Biol Chem* 277:2463–2467. <https://doi.org/10.1074/jbc.M105161200>
28. Lu TT, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, Mangelsdorf DJ (2000) Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol Cell* 6:507–515. [https://doi.org/10.1016/s1097-2765\(00\)00050-2](https://doi.org/10.1016/s1097-2765(00)00050-2)
29. Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, Galardi C, Wilson JG, Lewis MC, Roth ME (2000) A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol Cell* 6:517–526. [https://doi.org/10.1016/s1097-2765\(00\)00051-4](https://doi.org/10.1016/s1097-2765(00)00051-4)
30. Suzuki T, Kasahara M, Yoshioka H, K-i M, Umesono K (2003) LXXLL-related motifs in Dax-1 have target specificity for the orphan nuclear receptors Ad4BP/SF-1 and LRH-1. *Mol Cell Biol* 23:238–249. <https://doi.org/10.1128/mcb.23.1.238-249.2003>
31. Kamiya A, Kakinuma S, Onodera M, Miyajima A, Nakauchi H (2008) Prospero-related homeobox 1 and liver receptor homologue 1 coordinately regulate long-term proliferation of murine fetal hepatoblasts. *Hepatology* 48:252–264. <https://doi.org/10.1002/hep.22303>
32. Qin J, Gao D-m, Jiang Q-F, Zhou Q, Kong Y-Y, Wang Y, Xie Y-H (2004) Prospero-related homeobox (Prox1) is a corepressor of human liver receptor homologue-1 and suppresses the transcription of the cholesterol 7- α -hydroxylase gene. *Mol Endocrinol* 18:2424–2439. <https://doi.org/10.1210/me.2004-0009>
33. Stein S, Oosterveer MH, Matakic C, Xu P, Lemos V, Havinga R, Dittner C, Ryu D, Menzies KJ, Wang X, Perino A, Houten SM, Melchior F, Schoonjans K (2014) SUMOylation-dependent LRH-1/PROX1 interaction promotes atherosclerosis by decreasing hepatic reverse cholesterol transport. *Cell Metab* 20:603–613. <https://doi.org/10.1016/j.cmet.2014.07.023>
34. Bouchard MF, Taniguchi H, Viger RS (2005) Protein kinase A-dependent synergism between GATA factors and the nuclear receptor, liver receptor homologue-1, regulates human aromatase (CYP19) PII promoter activity in breast cancer cells. *Endocrinology* 146:4905–4916. <https://doi.org/10.1210/en.2005-0187>
35. Chanda D, Xie Y-B, Choi H-S (2010) Transcriptional corepressor SHP recruits SIRT1 histone deacetylase to inhibit LRH-1

- transactivation. *Nucleic Acids Res* 38:4607–4619. <https://doi.org/10.1093/nar/gkq227>
36. Chalkiadaki A, Talianidis I (2005) SUMO-dependent compartmentalization in promyelocytic leukemia protein nuclear bodies prevents the access of LRH-1 to chromatin. *Mol Cell Biol* 25:5095–5105. <https://doi.org/10.1128/MCB.25.12.5095-5105.2005>
 37. Whitby RJ, Stec J, Blind RD, Dixon S, Leesnitzer LM, Orband-Miller LA, Williams SP, Willson TM, Xu R, Zuercher WJ (2011) Small molecule agonists of the orphan nuclear receptors steroidogenic factor-1 (SF-1, NR5A1) and liver receptor homologue-1 (LRH-1, NR5A2). *J Med Chem* 54:2266–2281. <https://doi.org/10.1021/jm1014296>
 38. Bayrer JR, Wang H, Nattiv R, Suzawa M, Escusa HS, Fletterick RJ, Klein OD, Moore DD, Ingraham HA (2018) LRH-1 mitigates intestinal inflammatory disease by maintaining epithelial homeostasis and cell survival. *Nat Commun* 9:4055. <https://doi.org/10.1038/s41467-018-06137-w>
 39. Nissim S, Weeks O, Talbot JC, Hedgepeth JW, Wucherpfennig J, Schatzman-Bone S, Swinburne I, Cortes M, Alexa K, Megason S, North TE, Amacher SL, Goessling W (2016) Iterative use of nuclear receptor Nr5a2 regulates multiple stages of liver and pancreas development. *Dev Biol* 418:108–123. <https://doi.org/10.1016/j.ydbio.2016.07.019>
 40. Wagner M, Zollner G, Trauner M (2010) Nuclear receptor regulation of the adaptive response of bile acid transporters in cholestasis. *Semin Liver Dis* 30:160–177. <https://doi.org/10.1055/s-0030-1253225>
 41. D'Errico I, Moschetta A (2008) Nuclear receptors, intestinal architecture and colon cancer: an intriguing link. *Cell Mol Life Sci* 65:1523–1543. <https://doi.org/10.1007/s00018-008-7552-1>
 42. Annicotte J-S, Fayard E, Swift GH, Selander L, Edlund H, Tanaka T, Kodama T, Schoonjans K, Auwerx J (2003) Pancreatic-duodenal homeobox 1 regulates expression of liver receptor homolog 1 during pancreas development. *Mol Cell Biol* 23:6713–6724. <https://doi.org/10.1128/mcb.23.19.6713-6724.2003>
 43. Wagner RT, Xu X, Yi F, Merrill BJ, Cooney AJ (2010) Canonical Wnt/ β -catenin regulation of liver receptor homolog-1 mediates pluripotency gene expression. *Stem Cells* 28:1794–1804. <https://doi.org/10.1002/stem.502>
 44. Heng J-CD, Feng B, Han J, Jiang J, Kraus P, Ng J-H, Orlov YL, Huss M, Yang L, Lufkin T (2010) The nuclear receptor Nr5a2 can replace Oct4 in the reprogramming of murine somatic cells to pluripotent cells. *Cell Stem Cell* 6:167–174. <https://doi.org/10.1016/j.stem.2009.12.009>
 45. Kelly VR, Hammer GD (2011) LRH-1 and Nanog regulate Dax1 transcription in mouse embryonic stem cells. *Mol Cell Endocrinol* 332:116–124. <https://doi.org/10.1016/j.mce.2010.10.003>
 46. Van Der Flier LG, Clevers H (2009) Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol* 71:241–260. <https://doi.org/10.1146/annurev.physiol.010908.163145>
 47. Crosnier C, Stamatakis D, Lewis J (2006) Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. *Nat Rev Genet* 7:349–359. <https://doi.org/10.1038/nrg1840>
 48. Kostadinova F, Schwaderer J, Sebeo V, Brunner T (2014) Why does the gut synthesize glucocorticoids? *Ann Med* 46:490–497. <https://doi.org/10.3109/07853890.2014.932920>
 49. Saxena D, Safi R, Little-Ihrig L, Zeleznik AJ (2004) Liver receptor homolog-1 stimulates the progesterone biosynthetic pathway during follicle-stimulating hormone-induced granulosa cell differentiation. *Endocrinology* 145:3821–3829. <https://doi.org/10.1210/en.2004-0423>
 50. Duggavathi R, Volle DH, Matakis C, Antal MC, Messaddeq N, Auwerx J, Murphy BD, Schoonjans K (2008) Liver receptor homolog 1 is essential for ovulation. *Genes Dev* 22:1871–1876. <https://doi.org/10.1101/gad.472008>
 51. Oosterveer MH, Schoonjans K (2014) Hepatic glucose sensing and integrative pathways in the liver. *Cell Mol Life Sci* 71:1453–1467. <https://doi.org/10.1007/s00018-013-1505-z>
 52. Oosterveer MH, Matakis C, Yamamoto H, Harach T, Moullan N, van Dijk TH, Ayuso E, Bosch F, Postic C, Groen AK, Auwerx J, Schoonjans K (2012) LRH-1-dependent glucose sensing determines intermediary metabolism in liver. *J Clin Invest* 122:2817–2826. <https://doi.org/10.1172/JCI62368>
 53. Luo Y, C-p L, Tall AR (2001) The orphan nuclear receptor LRH-1 potentiates the sterol-mediated induction of the human CETP gene by liver X receptor. *J Biol Chem* 276:24767–24773. <https://doi.org/10.1074/jbc.M100912200>
 54. Schoonjans K, Annicotte JS, Huby T, Botrugno OA, Fayard E, Ueda Y, Chapman J, Auwerx J (2002) Liver receptor homolog 1 controls the expression of the scavenger receptor class B type I. *EMBO Rep* 3:1181–1187. <https://doi.org/10.1093/embo-reports/kvf238>
 55. Lee YK, Schmidt DR, Cummins CL, Choi M, Peng L, Zhang Y, Goodwin B, Hammer RE, Mangelsdorf DJ, Kliewer SA (2008) Liver receptor homolog-1 regulates bile acid homeostasis but is not essential for feedback regulation of bile acid synthesis. *Mol Endocrinol* 22:1345–1356. <https://doi.org/10.1210/me.2007-0565>
 56. Out C, Hageman J, Bloks VW, Gerrits H, Sollewijn Gelpke MD, Bos T, Havinga R, Smit MJ, Kuipers F, Groen AK (2011) Liver receptor homolog-1 is critical for adequate up-regulation of Cyp7a1 gene transcription and bile salt synthesis during bile salt sequestration. *Hepatology* 53:2075–2085. <https://doi.org/10.1002/hep.24286>
 57. Mamrosh JL, Lee JM, Wagner M, Stambrook PJ, Whitby RJ, Sifers RN, Wu SP, Tsai MJ, Demayo FJ, Moore DD (2014) Nuclear receptor LRH-1/NR5A2 is required and targetable for liver endoplasmic reticulum stress resolution. *Elife* 3:e01694. <https://doi.org/10.7554/eLife.01694>
 58. Coste A, Dubuquoy L, Barnouin R, Annicotte J-S, Magnier B, Notti M, Corazza N, Antal MC, Metzger D, Desreumaux P (2007) LRH-1-mediated glucocorticoid synthesis in enterocytes protects against inflammatory bowel disease. *Proc Natl Acad Sci USA* 104:13098–13103. <https://doi.org/10.1073/pnas.0702440104>
 59. Taves MD, Gomez-Sanchez CE, Soma KK (2011) Extra-adrenal glucocorticoids and mineralocorticoids: evidence for local synthesis, regulation, and function. *Am J Physiol Endocrinol Metab* 301:E11–E24. <https://doi.org/10.1152/ajpendo.00100.2011>
 60. Garg PK, Narayana D (2016) Changing phenotype and disease behaviour of chronic pancreatitis in India: evidence for gene-environment interactions. *Glob Health Epidemiol Genomics* 1:e17. <https://doi.org/10.1017/ghg.2016.13>
 61. Prakash VB (2019) Changing demography of pancreatitis patients in India—A hospital based study. *Acta Sci Gastrointest Disord* 2:08–11. <https://doi.org/10.1017/ghg.2016.13>
 62. Murtaugh LC, Keefe MD (2015) Regeneration and repair of the exocrine pancreas. *Annu Rev Physiol* 77:229–249. <https://doi.org/10.1146/annurev-physiol-021014-071727>
 63. Cobo I, Martinelli P, Flandez M, Bakiri L, Zhang M, Carrillo-de-Santa-Pau E, Jia J, Sanchez-Arevalo Lobo VJ, Megias D, Felipe I, Del Pozo N, Millan I, Thommesen L, Bruland T, Olson SH, Smith J, Schoonjans K, Bamlet WR, Petersen GM, Malats N, Amundadottir LT, Wagner EF, Real FX (2018) Transcriptional regulation by NR5A2 links differentiation and inflammation in the pancreas. *Nature* 554:533–537. <https://doi.org/10.1038/nature25751>
 64. Benod C, Vinogradova MV, Jouravel N, Kim GE, Fletterick RJ, Sablin EP (2011) Nuclear receptor liver receptor homologue 1 (LRH-1) regulates pancreatic cancer cell growth and proliferation.

- Proc Natl Acad Sci USA 108:16927–16931. <https://doi.org/10.1073/pnas.1112047108>
65. Flandez M, Cendrowski J, Cañamero M, Salas A, Del Pozo N, Schoonjans K, Real FX (2014) Nr5a2 heterozygosity sensitises to, and cooperates with, inflammation in KRasG12V-driven pancreatic tumourigenesis. *Gut* 63:647–655. <https://doi.org/10.1136/gutjnl-2012-304381>
66. Amundadottir LT (2016) Pancreatic cancer genetics. *Int J Biol Sci* 12:314–325. <https://doi.org/10.7150/ijbs.15001>
67. Sun YM, Zheng S, Chen X, Gao F, Zhang J (2020) Lower Nr5a2 level downregulates the β -Catenin and TCF-4 expression in caerulein-induced pancreatic inflammation. *Front Physiol* 10:1549. <https://doi.org/10.3389/fphys.2019.01549>
68. Macchini M, Chiaravalli M, Zanon S, Peretti U, Mazza E, Gianni L, Reni M (2019) Chemotherapy in elderly patients with pancreatic cancer: efficacy, feasibility and future perspectives. *Cancer Treat Rev* 72:1–6. <https://doi.org/10.1016/j.ctrv.2018.10.013>
69. Petersen GM, Amundadottir L, Fuchs CS, Kraft P, Stolzenberg-Solomon RZ, Jacobs KB, Arslan AA, Bueno-de-Mesquita HB, Gallinger S, Gross M, Helzlsouer K, Holly EA, Jacobs EJ, Klein AP, LaCroix A, Li D, Mandelson MT, Olson SH, Risch HA, Zheng W, Albanes D, Bamlet WR, Berg CD, Boutron-Ruault MC, Buring JE, Bracci PM, Canzian F, Clipp S, Cotterchio M, de Andrade M, Duell EJ, Gaziano JM, Giovannucci EL, Goggins M, Hallmans G, Hankinson SE, Hassan M, Howard B, Hunter DJ, Hutchinson A, Jenab M, Kaaks R, Kooperberg C, Krogh V, Kurtz RC, Lynch SM, McWilliams RR, Mendelsohn JB, Michaud DS, Parikh H, Patel AV, Peeters PH, Rajkovic A, Riboli E, Rodriguez L, Seminara D, Shu XO, Thomas G, Tjonneland A, Tobias GS, Trichopoulos D, Van Den Eeden SK, Virtamo J, Wactawski-Wende J, Wang Z, Wolpin BM, Yu H, Yu K, Zeleniuch-Jacquotte A, Fraumeni JF Jr, Hoover RN, Hartge P, Chanock SJ (2010) A genome-wide association study identifies pancreatic cancer susceptibility loci on chromosomes 13q22.1, 1q32.1 and 5p15.33. *Nat Genet* 42:224–228. <https://doi.org/10.1038/ng.522>
70. von Figura G, Morris JPT, Wright CV, Hebrok M (2014) Nr5a2 maintains acinar cell differentiation and constrains oncogenic Kras-mediated pancreatic neoplastic initiation. *Gut* 63:656–664. <https://doi.org/10.1136/gutjnl-2012-304287>
71. Ramsey ML, Conwell DL, Hart PA (2017) Complications of chronic pancreatitis. *Dig Dis Sci* 62:1745–1750. <https://doi.org/10.1007/s10620-017-4518-x>
72. Seimiya T, Otsuka M, Iwata T, Tanaka E, Suzuki T, Sekiba K, Yamagami M, Ishibashi R, Koike K (2018) Inflammation and de-differentiation in pancreatic carcinogenesis. *World J Clin Cases* 6:882–891. <https://doi.org/10.12998/wjcc.v6.i15.882>
73. Midha S, Chawla S, Garg PK (2016) Modifiable and non-modifiable risk factors for pancreatic cancer: a review. *Cancer Lett* 381:269–277. <https://doi.org/10.1016/j.canlet.2016.07.022>
74. Yadav D, Lowenfels AB (2013) The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology* 144:1252–1261. <https://doi.org/10.1053/j.gastro.2013.01.068>
75. Hammad AY, Ditillo M, Castanon L (2018) Pancreatitis. *Surg Clin* 98:895–913. <https://doi.org/10.1016/j.suc.2018.06.001>

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