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Review

New Insights into the Diverse Functions of the NR2F Nuclear Orphan Receptor Family

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Abstract

Following gene expansion during evolution, today's phylogenetic tree of the NR2F family of nuclear orphan receptors in mammals is represented by three different isoforms: NR2F1, NR2F2, and NR2F6. Structural analysis of the NR2F family members has revealed that NR2F1 and NR2F2 are closely related and grouped together apart from NR2F6, which is more divergent in its biochemical characteristics. In this review, we highlight current knowledge on the cellular functions of NR2F family members. NR2F family members have been reported to be causally involved in carcinogenesis. Mechanistically, NR2F proteins are localized in the nucleus, where they bind to target DNA enhancer sequences and have been implicated in the regulation of de novo gene transcription, though this is not sufficiently understood. Based on apparently divergent and non-uniform expression patterns of the NR2F isoforms in different tissues and cell types, non-redundant functions of the individual family members appear to exist. Notably, NR2F2 appears to be more closely related functionally to NR2F6 than NR2F1. Along these lines, NR2F2 and NR2F6 have been reported to be involved in cellular neoplasia. Furthermore, enhanced expression of NR2F isoforms has been established as prognostic biomarkers in various cancer entities. Therefore, it is tempting to speculate that NR2F isoforms represent innovative targets for therapeutic intervention in defined types of cancer. Thus, NR2F family nuclear receptors can be viewed as gatekeepers balancing cell type-specific regulation of proliferation and the suppression of terminal differentiation in health and disease.

Keywords: nuclear receptors; NR2F family; NR2F isoform-specific functions; development; cell differentiation; metabolism; carcinogenesis

1. Introduction

Nuclear receptors (NRs) are ligand-activated transcription factors involved in the regulation of a wide array of physiological and developmental processes. The NR superfamily consists of 48 transcription factors in humans, including the receptors for steroid hormones, thyroid hormones, cholesterol metabolites, and lipophilic vitamins. However, approximately half of the NRs are categorized as orphan receptors because their ligands are not known [1]. As the name suggests, NRs are located in the nucleus, where they function as transcriptional regulators with direct DNA binding activity, regulating gene expression. Due to their excellent druggability, NRs are promising candidates as novel therapeutic targets for a multitude of diseases, including cancer [2]. NRs exhibit a characteristic modular structure that is composed of five to six homologous domains designated A to F (from the N-terminal to the C-terminal end). This nomenclature is based on the region of conserved sequence and function. The defining features of NRs are the DNA binding domain (DBD; region C) and ligand binding domain (LBD; region E), which are both highly conserved. They are the two most important regions and can function independently from each other. The variable N-terminal A/B region, as well as the D region, are less conserved. The C-terminal F region does not exist in all receptors, and its function is not well understood [3]. The first NR was described in 1985 [4], and the NR superfamily is classified according to their evolutionary distance into six subfamilies, numbered from 1 to 6. These six subfamilies are further divided into several groups, starting with the letter A and further specified by an additional number [3,5].

The NR2F family, also known as the nuclear orphan receptors of the chicken ovalbumin upstream promoter transcription factor (COUP-TF) family, consists of three members: NR2F1 (synonyms: COUP-TFI, EAR-3), NR2F2 (synonyms: COUP-TFII, ARP-1), and NR2F6 (synonyms: COUP-TFIII, EAR-2) [5–8]. Among the NR2F family members, NR2F1 and NR2F2 have the highest homology, especially in the functionally important DBD and LBD, with a homology of 98 percent and 96 percent, respectively [7]. The third NR2F family member, NR2F6, is more divergent but still functionally closely related [9]. The NR2F family members homo- or heterodimerize with retinoid X receptor (RXR/NR2B1) and other NRs, which results in binding to a variety of response elements containing imperfect AGGTCA direct or inverted repeats with various spacing on the cognate DNA sequence. All of the members of the NR2F family are orphan receptors because endogenous ligands have not yet been identified [10].

Table 1. Function of NR2F family members in angiogenesis.

Family member	Area	Effect	Ref.
NR2F2	Lymphatic vessel formation in constitutive knockout mice	Venous and lymphatic vessel formation defects	[25]
NR2F2	Notch pathway inhibitor	FoxC1 and NR-1 binding upstream of Notch	[28]
		Hey2 binding downstream of Notch	[28]
NR2F2	Repressor of artery-specific genes	Reduced NR2F2 expression associated with higher artery marker expression	[29,30]
		Promotes cell proliferation and sprouting in endothelial cells	[29,30]
NR2F2	Maintenance of venous identity	High NR2F2 expression in endothelial progenitor cells (EPCs) in vein patch	[31,32]
		Hypoxia-induced NR2F2 downregulation	[32]
		Regulatory function on VEGF-C and G, VEGF-R3, and NP-2 expression	[33,34]
		Humans: NR2F2-mediated activation of cell cycle genes, activation of vein-specific enhancers, repression of artery enhancers	[35]
NR2F2	Lymphatic endothelial cell fate regulator	NR2F2 and Sox18 induce Prox1 expression	[36]
		NR2F2-Prox1 heterodimers allow for lymph marker expression	[36–40]

2. NR2F Family Functionality

2.1 Cortical Development and Neurogenesis

NR2F1 is regarded as one of the main transcriptional regulators governing cortical arealization, cell-type specification, and maturation. Multi-faceted functions of NR2F1 in the development of different mouse brain structures, including the neocortex, hippocampus, and ganglionic eminences, have been observed [11]. Other functions of NR2F1 include regulating migration [12,13], controlling temporal identity specification of neuronal progenitor cells [14,15], and constituting area-specific identity in progenitors and neurons [16–18]. In humans, NR2F1 haploinsufficiency has been associated with the rare disease Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS), a complex neurodevelopmental disease affecting intellectual ability and resulting in optic atrophy [19,20]. The *Nr2f1* heterozygous knockout mouse model exhibits some of the neurological symptoms of BBSOAS and, mechanistically, impaired hippocampal synaptic plasticity has been observed. This suggests that a deficit or alteration in hippocampal synaptic plasticity contributes to the intellectual disability symptoms present in BBSOAS [21].

2.2 Development and Differentiation

In vertebrates, NR2F1 and NR2F2 are expressed during early development. In general, NR2F1 is predominantly expressed in the developing peripheral and central nervous system, whereas NR2F2 is present in the mesenchymal area of the internal organs. In mice, homozygous deletions of NR2F1 result in perinatal lethality due to defects in the central nervous system. In contrast, mice with homozygous deletions of NR2F2 die due to growth retardation in the vasculature of the head, spine, and heart [22]. Evidently, NR2F2 is involved in tissue homeostasis and maintenance, functioning as a major regulator of cell differentiation and angiogenesis [23–25]. Moreover, studies with genetically modified mouse models have shown major regulatory functions of NR2F2 in the development of several tissues and organs, including the kidney, stomach, and diaphragm [23,26,27].

2.3 Angiogenesis

Mice deficient in NR2F2 exhibit defects in angiogenesis, particularly in the appearance of venous and lymphatic vessels, which suggests the involvement of NR2F2 in angiogenesis [25] (Table 1, Ref. [25,28–40]). The major drivers of this process are the Notch signaling pathway and vascular endothelial growth factor (VEGF), alongside the BMP/TGF-β and Hedgehog (HH) pathways [41,42]. Notch signaling directly or indirectly modulates angiogenesis through receptors for VEGF [43]. During angiogenesis, vessel cells differentiate into the venous phenotype, but Notch signals direct the cells toward the artery phenotype [44]. NR2F2 inhibits the Notch pathway, acting both upstream and downstream of Notch. Upstream, NR2F2 directly binds to FoxC1 and NR-1, which reduces the expression of these targets, whereas the downstream target of NR2F2 is Hey2 [28]. In the Notch pathway, NR2F2 represses the transcription of artery-specific genes. In endothelial cells with reduced NR2F2 expression, lower proliferation and higher expression of artery markers has been observed, both of which have been observed to occur in response to Notch activation. In endothelial cells, NR2F2 promotes cell proliferation and cell sprouting, which is mediated by higher expression of E2F1 due to regulation by NR2F2 and SP1 [29,30].

In the vein patch, endothelial progenitor cells (EPCs) express high levels of vein markers, such as NR2F2, whereas arterial markers Dll-4 and Hey2 are expressed at lower levels [31,32]. The oxygen sensor HIFα induces ex-

Table 2. Functions of NR2F family members in carcinogenesis.

Family member	Area	Effect	Ref.
NR2F1	Expression in tumor cells	Associated with tumoral phenotype	[49–51]
		NR2F1 expression lower than in normal cells	[52–54]
NR2F2	Angiogenesis regulator within the tumor microenvironment	Conditional ablation of NR2F2 compromises neoangiogenesis and suppresses tumor growth	[55]
NR2F2	Facilitator of prostate tumorigenesis	NR2F2-mediated inhibition of TGF-β-induced growth barrier and inhibitor of mothers against decapentaplegic homolog 4 (SMAD4)-dependent transcription	[56]
		Correlation of NR2F2 expression with disease progression	[56]
NR2F2	Involvement in estrogen receptor expression	NR2F2 expression associated with loss of estrogen receptor expression	[57]
NR2F6	Regulator in leukemia	Expression significantly induced in leukemia	[58]
		Tumor suppressor in mixed-lineage leukemia acute lymphocytic leukemia 1-fused gene from chromosome 4 (MLL-AF4) acute leukemia	[59]
		Patients with missense NR2F6 mutations in mast cell leukemia with associated hematological neoplasm (MCL-AHN) leukemia	[60]
NR2F6	Prognostic marker	Muscle-invasive bladder cancer	[61]
		Head and neck squamous cell carcinoma	[62]
		Early-stage cervical cancer	[63]
		Ovarian cancer	[65,66]
NR2F6	Colorectal cancer	Expression of NR2F6 significantly induced in colorectal cancer	[64]
NR2F6	Involvement in liver cancer	Expression is implicated in progression of hepatocellular carcinoma	[67]
		Expression contributes to rapid progression of hepatoblastoma	[68]
NR2F6	Non-small cell lung cancer	Inhibition of NR2F6 expression results in suppression of proliferation, migration, and invasion of cancer cells	[69]
		Single nucleotide polymorphism in nonsmall cell lung cancer (NSCLC) patients associated with better overall survival	[70]

pression of Notch ligand Dll-4 and Notch target genes Hey1 and Hey2 in EPCs in response to hypoxia, which results in reduced NR2F2 expression. In addition, Hey2 decreases HIFα-mediated gene expression. Apparently, a negative feedback loop avoids exceeding hypoxic gene induction, and oxygen availability plays an important role in the fate of endothelial cells regulating NR2F2 and Notch expression [32].

In addition to the involvement of NR2F2 in Notch signaling, NR2F2 also induces vein and lymph node identity by regulating the expression of VEGF-C and -D, as well as VEGF-R3 and NP-2 [33,34]. The expression of NR2F2 in vein cells depends on the transcription factor brahma-related gene 1 (BRG1) [45]. Interestingly, BRG1 expression has also been reported to be necessary for the expression of Notch ligands [46,47].

In a study that focused on identifying regulatory elements in the human genome responsible for controlling artery and vein gene expression, several thousand artery- and vein-specific regulatory elements were identified. This genomic characterization of endothelial enhancers exhibited overrepresentation of NR2F2 sites in vein-specific enhancers, suggesting a direct role in promoting vein identity and a multifunctional role of NR2F2 in the regulation of arteriovenous gene expression. In particular, NR2F2 has been shown to regulate three distinct aspects of arteriovenous identity. First, in accordance with previous studies, they observed that NR2F2 directly activates enhancer elements flanking cell cycle genes to drive their expression. Second, NR2F2 appears to be necessary for direct activation of vein-specific enhancers and their associated genes. Third, NR2F2 directly represses artery enhancers in venous cells, such as Hey2, preventing their activation. Apparently, NR2F2 functions in multiple roles to maintain venous identity [35].

In lymphatic vessels, sustained expression of the transcription factor Prospero homeobox protein 1 (Prox1) is required to maintain the lymphatic identity in adults. Mice deficient in *Prox1* do not develop lymphatic structures [48]. The transcription factor SRY-related gene (Sox) 18 is expressed before Prox1 and required for Prox1 expression during development. However, Sox18 alone is not enough for the induction of Prox1 expression. Presumably, some arterial-specific gene hinders Prox1 induction. Alternatively, some vein-specific factors could cooperate with Sox18 to induce Prox1. One of those factors might be NR2F2 since it was observed that NR2F2 acts jointly with Sox18 to induce Prox1 expression in embryonic veins [36]. NR2F2 binds directly to a conserved site within the upstream regulatory region of the *Prox1* promoter and forms

heterodimers with Prox1 [36,37]. Though NR2F2 homodimers inhibit the Notch pathway, NR2F2 heterodimers with Prox1 are not able to accomplish this and, thus, Notch effectors are partially expressed in lymphatic vessels. Furthermore, heterodimers allow for the expression of lymph markers, such as VEGF-R3, which binds to VEGF-C and induces the expression of cyclin E1 [37–39]. Further evidence of the importance of NR2F2 in lymphatic specification is the observation that NR2F2 and Prox1 are suppressed by Hey1 and Hey2. Moreover, the expression of Notch effectors changes lymph cells into arterial-like cells. This suggests that lymph cells are extremely plastic and external stimuli could give rise to all types of endothelial cells. This is reinforced by the observation that all three master regulators of endothelial specification, namely Notch, NR2F2, and Prox1, are co-expressed in lymph cells, indicating a cross-control mechanism between these cell fate regulators. Consequently, a slight change in the expression of these regulators may lead to reprogramming of the lymphatic endothelial cell fate [40].

2.4 Carcinogenesis

The role of the NR2F family in cancer progression appears to be diverse (Table 2, Ref. [49–70]). Depending on the cell type and biological processes examined, the NR2F family members have been proposed to act negatively or positively on cancer progression [49]. In some tumors, NR2F1 is re-expressed in the process of dedifferentiation and associated with the tumor phenotype [50], with higher expression compared to normal tissue samples [49–51]. In contrast, some cancer types have been reported to have lower NR2F1 expression compared to normal tissue [52,53]. High NR2F1 expression is linked to increased cell proliferation and migration in breast cancer [50]. In contrast, high NR2F1 expression appears to function as a cell cycle break in other tumor types, such as prostate cancer or head and neck squamous cell carcinoma (HNSCC), which causes long-term quiescence in dormant cancerous cells [54]. One study reported the discovery of an NR2F1-specific agonist that activates dormancy programs in malignant cells, suppressing metastasis by inducing cancer cell dormancy [71]. Thus, even in tumor tissues, NR2F1 apparently has a multitude of functions; the expression levels and molecular roles in cancer development and progression differ based on the context and tissue type and whether cell proliferation or migration is affected [11].

As mentioned above, NR2F2 plays an important role in angiogenesis. This is also relevant in the context of tumorigenesis and tumor progression because tumor growth depends on nutrients and oxygen supply via the vasculature through angiogenesis [55,72]. One study described NR2F2 as a major regulator of angiogenesis within the tumor microenvironment. Conditional ablation of NR2F2 in adults massively compromised neoangiogenesis and suppressed tumor growth in xenograft mouse models. The same study observed that the absence of NR2F2 in a spontaneous mammary-gland tumor model resulted in impaired tumor growth and tumor metastasis. Overall, it appears that NR2F2 is an important regulator of the pathological neovascular response [55].

Mutations in phosphate and tensin homologue (PTEN) have frequently been observed in human prostate cancer [73,74]. PTEN loss can lead to upregulation of TGF-β signaling, which may result in the formation of a growth barrier to limit prostate cancer progression [75]. One study described how NR2F2 inhibits the TGF-β-induced growth barrier, facilitating prostate tumorigenesis. Mechanistically, NR2F2 is a major regulator and inhibitor of SMAD4-dependent transcription, which overrules the TGF-β-dependent checkpoint for PTEN-null indolent tumors. In the mouse prostate epithelium, overexpression of NR2F2 cooperates with PTEN deletion to reinforce the progression of malignancy and generate metastasis-prone tumors. A patient sample analysis revealed that NR2F2 expression or activity substantially correlates with tumor recurrence and disease progression. In addition, NR2F2 expression is inversely associated with TGF-β signaling. Taken together, these results suggest that NR2F2 is essential for destruction of the TGF-β-dependent barrier observed in PTEN-mutant prostate cancer [56].

In mammary cancer cell lines, NR2F2 expression is associated with loss of estrogen receptor expression [57]. Estrogen receptor expression serves as a major indicator of the hormone-dependent cancer differentiation state. Tumors without estrogen receptor expression, so-called ER-negative tumors, are histologically less differentiated and have superior metastatic potential [76]. Interestingly, one study observed that estrogen receptor-positive breast cancer cells have increased NR2F2 expression, whereas estrogen receptor-negative cells expressed a low amount of NR2F2 [77].

The third family member, NR2F6, has been reported by various studies to be involved in carcinogenesis and progression. For example, NR2F6 expression is significantly induced in leukemia [58]. Mechanistically, the same group reported that NR2F6 inhibits hematopoietic cell differentiation and induces myeloid dysplasia [78]. One study focusing on t (4;11) MLL-AF4 acute leukemia, a specific form of acute lymphoblastic leukemia characterized by the MLL-AF4 fusion gene associated with poor prognosis, identified NR2F6 as a novel tumor suppressor of MLL-AF4$^+$ leukemia [59]. In mast cell leukemia with associated hematological neoplasm (MCL-AHN), a rare and very aggressive form of leukemia, an in-depth study of five cases discovered a novel and identical NR2F6 missense variant (NR2F6 p. P132A) in two of the patients [60].

NR2F6 has been proposed as a prognostic biomarker of muscle-invasive bladder cancer (MIBC) and HNSCC [61,62]. NR2F6 expression has also been reported to correlate with pelvic lymph node metastasis and overall poorer

Table 3. Functions of NR2F family members in metabolism.

Family member	Area	Effect	Ref.
NR2F2	Adipose tissue regulation in NR2F2 heterozygous mice	Less white adipose tissue, no obesity when fed high-fat diet	[82]
NR2F2	Wnt/β-catenin signaling	Reduction of NR2F2 expression increases Wnt signaling	[82]
		Activation of NR2F2 expression due to Wnt/β-catenin signaling	[83]
		Potentially important β-catenin cofactor under hypoxic conditions	[84]
NR2F2	Adipocyte differentiation	Necessary for adipocyte differentiation	[83]
NR2F2	Insulin secretion	Tissue-specific knockdown of NR2F2 in β-cells results in abnormal pattern of insulin secretion	[85]
		Reciprocal regulation of insulin and NR2F2 due to forkhead box protein O1 (FOXO1)	[85]
		Humans: single nucleotide polymorphism observed with lower blood insulin concentrations	[86]
NR2F2	Liver function	Expression in liver inhibited by glucose and insulin	[80,85]
		Induces upregulation of gluconeogenic enzymes and β-oxidation pathway genes during fasting	[87]
		Interaction with glucocorticoid receptor	[81]
NR2F2	Mitochondrial dysfunction	Overexpression causes defects in electron transport chain activity and higher reactive oxygen species (ROS) production	[88]
NR2F2	Skeletal muscle and heart muscle	Levels in skeletal muscle regulate Glut4 expression	[89]
		Important for myogenesis in skeletal muscle	[90]
		Overexpression reduces cardiac performance and results in cardiomyopathy and heart failure	[25,88]
NR2F2	Fibrosis	Increased NR2F2 expression in myofibroblasts	[92]
NR2F6	Adipocyte differentiation	Downregulation of NR2F6 inhibits adipogenesis	[91]
NR2F6	Liver function	NR2F6 upregulation in patients with non-alcoholic fatty liver disease	[92]
		Beef cattle: key regulator of hepatic inflammatory response	[93]

prognosis in early-stage cervical cancer [63]. NR2F6 expression is significantly induced in colorectal cancer [64]. NR2F6 upregulation has also been detected in ovarian cancer and is associated with significantly worse overall survival [65]. In breast cancer, one study reported the involvement of NR2F6 in regulating the docetaxel chemosensitivity [66]. Furthermore, overexpression of NR2F6 was described to promote the chemoresistance of epithelial ovarian cancer by activating the Notch3 signaling pathway. The same study proposed NR2F6 as a biomarker for identifying patients who are likely to respond to therapy with gamma-secretase inhibitors, which inhibit Notch signaling [79].

In hepatocellular carcinoma (HCC), NR2F6 expression is implicated in disease progression. Knocking out NR2F6 inhibits the growth, migration, and invasion of HCC cells [67]. Corresponding to these observations, NR2F6 upregulation has been reported to be higher in hepatoblastoma than non-cancer livers, and NR2F6 expression was suggested to contribute to the rapid progression of residual liver tumor in hepatoblastoma [68].

NR2F6 also plays a role in the progression of non-small cell lung cancer (NSCLC). Mechanistically, the microRNA miR-142-3p directly inhibits NR2F6 expression, resulting in suppression of proliferation, migration, and invasion of cancer cells [69]. In accordance with this observation, one study reported that an analysis of single nucleotide polymorphisms at miRNA target sites in 782 early-stage NSCLC patients revealed that patients carrying the NR2F6 rs2288539 TT genotype have significantly better overall survival than patients with the NR2F6 rs2288539 CC or CT genotypes [70].

Evidently, NR2F family members can act as promoters or inhibitors of phenotypic modifications during cancer development and progression depending on the cellular context, expression levels, or possibly other transcription factors and signaling pathways [49].

2.5 Metabolism

The expression of NR2F2 in metabolic tissues was described for the first time about 20 years ago [80]. Since then, numerous studies have elucidated the role of NR2F2 in various metabolic systems, such as adipogenesis, lipid metabolism, insulin secretion, and hepatic gluconeogenesis (Table 3, Ref. [25,80–93]) [81]. NR2F2 heterozygous mice are characterized by less white adipose tissue than wild-type mice fed a high-fat diet and maintain a lean body mass phenotype. These heterozygous mice do not develop obesity. Mechanistically, they have reduced expression of NR2F2 in 3T3L1 cells, leading to increased Wnt signaling, which is known to be a repressive factor in adipogenesis [82]. Furthermore, Wnt/β-catenin signaling activates the expression of NR2F2. The Wnt signaling cascade acts though NR2F2

Table 4. NR2F6 functions in immune cells.

Cell type	Effect	Ref.
Macrophages	Mice: transcriptional repressor of cytokines	[103]
	Humans: transcriptional activator of chemokines	[103]
B cells	*Nr2f6* deficiency leads to accumulation of germinal center B cells secondary to follicular T helper cells	[104]
Plasma cells	*Nr2f6* deficiency leads to accumulation of plasma cells	[104]
Follicular T helper cells	*Nr2f6* deficiency leads to accumulation of follicular T helper cells	[104]
Autoimmunity via T-cell functions	Loss of *Nr2f6* exacerbates experimental autoimmune encephalomyelitis (EAE) due to negative regulation of *Il17a* expression by a direct NFAT and/or retinoic acid receptor-related orphan receptor C (RORC) antagonism	[105,106]
Cancer surveillance via T-cell functions	*Nr2f6*-deficient mice exhibit tumor growth inhibition benefit due to increased CD4 and CD8 T-cell infiltration leading to overall survival benefit	[98,100]
	In approximately 50% of human NSCLC biopsies, NR2F6 upregulation in tumor-infiltrating T lymphocytes (TILs) presumably contributes to the observed T-cell exhaustion	[100]

to activate PPARγ, the expression of which is necessary for adipogenesis. The same study showed that NR2F2 is necessary for adipocyte differentiation [83]. In the context of the Wnt/β-catenin and NR2F2 interaction, one study suggested that NR2F2 could be an important β-catenin cofactor under hypoxic conditions [84].

NR2F2 appears to be important for β cells and insulin secretion, and negative regulation of NR2F2 expression by glucose and insulin has been observed *in vitro* and *in vivo*. Tissue-specific knockdown of NR2F2 in β cells from mouse pancreas tissue results in an abnormal pattern of insulin secretion. In the presence of low glucose, insulin secretion is higher, but it is significantly decreased in response to glucose stimulation. Reciprocal regulation of insulin and NR2F2 has been described, which was accomplished by the forkhead box protein O1 (FOXO1) transcription factor [85]. NR2F2 heterozygous mice exhibit markedly improved glucose tolerance because of increased peripheral tissue insulin sensitivity [82]. An important finding regarding the insulin and NR2F2 interaction in humans was provided by a study that found a single nucleotide polymorphism (rs-3743462) in the distal glucose-responsive NR2F2 promoter; people with this polymorphism had lower blood insulin concentrations due to reduced NR2F2 expression [86].

Analogous to the pancreas, expression of NR2F2 in the liver is inhibited by glucose and insulin *in vitro* and *in vivo* [80,85]. NR2F2 expression in mice is upregulated under fasting conditions and downregulated upon re-feeding. During fasting, NR2F2 has been suggested to be involved in hepatic glucose production via upregulation of gluconeogenic enzymes and β-oxidation pathway genes [87]. In addition, the ability of NR2F2 to interact with the glucocorticoid receptor is important to the coordinated regulation of carbohydrate and lipid metabolism in adipose tissue and liver [81]. Furthermore, overexpression of NR2F2 results in mitochondrial dysfunction, impacting crucial metabolic pathways involving glucose uptake due to altered expression of glucose transporter type 4 (*Glut4*) and glycolysis due to altered expression of hexokinase-2 (*Hk2*) and phosphofructokinase (*Pfkm*). NR2F2 overexpression in mice also causes severe defects in electron transport chain activity and higher production of reactive oxygen species (ROS). As a result, a highly oxidizing environment is generated, with negative effects, such as protein oxidation, lipid peroxidation, and DNA damage [88]. Taken together, these results clearly highlight the role of NR2F2 as a regulator of metabolic homeostasis [81].

NR2F2 is expressed in multiple tissues and organs; therefore, its role in metabolism may not be restricted to the liver and pancreas [94]. Skeletal muscle is one of the most metabolically active tissues, and it is necessary to regulate the high energy demand via metabolic processes [95]. SiRNA-mediated knockdown of NR2F2 expression in mouse C2C12 myoblast cells reduces important genes implicated in the fatty acid β-oxidation pathway, thermogenesis, and cholesterol transport [96]. Furthermore, NR2F2 levels in skeletal muscle regulate *Glut4* expression levels [89]. NR2F2 plays an important role in myogenesis [90].

However, the involvement of NR2F2 is not limited to skeletal muscle cells; it is also observed in heart muscle cells. One study reported that overexpression of NR2F2 in mice markedly reduces cardiac performance [25]. Although evidence supports NR2F2 being crucial for the correct function and development of the cardiovascular system, its overexpression appears to be detrimental and may increase the risk of heart diseases. In mice, overexpression of NR2F2 in the myocardium results in dilated cardiomyopathy and heart failure [88].

A connection between metabolic disturbance and the development of fibrosis has been described in recent studies. One study reported increased NR2F2 expression in myofibroblasts from human fibrotic kidneys, lungs, kidney organoids, and mouse kidneys after injury. Genetic attenuation of NR2F2 in mice mitigates injury-induced kidney fi-

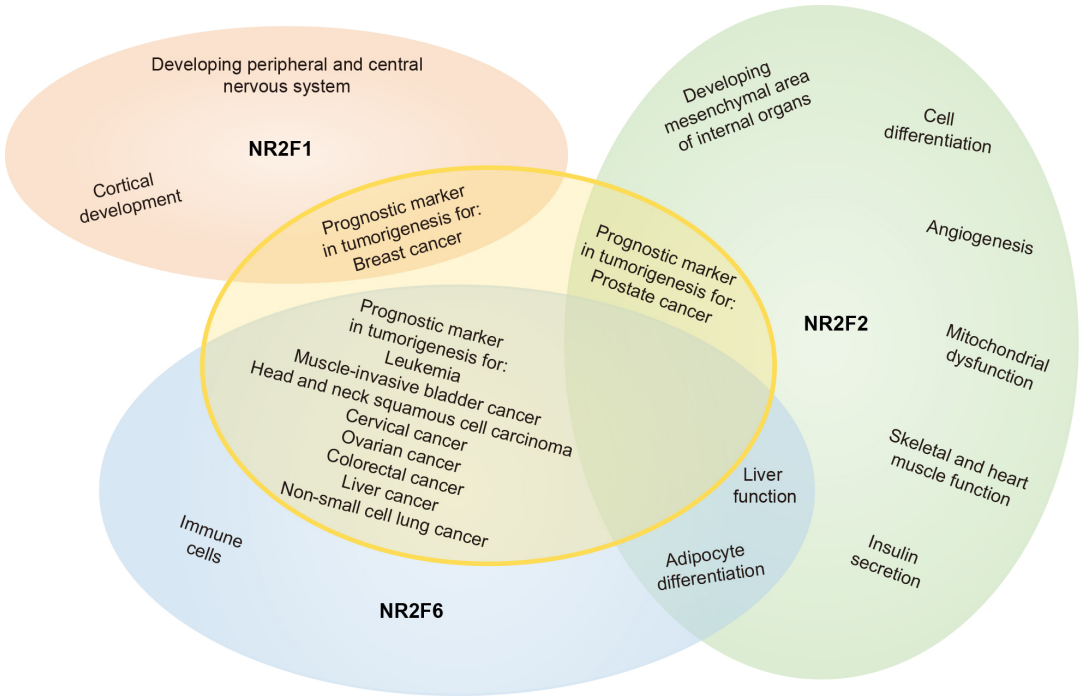

Fig. 1. Schematic overview of NR2F nuclear orphan receptor family functionality. A summary of the functions of NR2F family members in a broad range of areas. Though NR2F1 and NR2F2 are more structurally related to each other compared to NR2F6, it appears that NR2F2 and NR2F6 are more closely related functionally. An extensive summary of the functions of NR2F1, NR2F2, and NR2F6 can be found in Tables 1,2,3,4.

brosis. Mechanistically, suppression of fatty acid oxidation and enhancement of glycolysis pathways were the result of NR2F2 overexpression in fibroblasts [97].

NR2F6 has been implicated in metabolic regulation and has been reported to play a role in obesity control, as NR2F6 has been observed to be required for adipocyte differentiation and that downregulation of NR2F6 inhibits adipogenesis [91]. One study investigated the function of NR2F6 in the context of hepatic triglyceride homeostasis and reported NR2F6 as an important regulator and a causal factor in the development of non-alcoholic fatty liver disease. Mechanistically, the fatty acid translocase CD36, as a transcriptional target of NR2F6, has been implicated in this process. Furthermore, NR2F6 is upregulated in the livers of obese mice and patients with non-alcoholic fatty liver disease [92].

Taken together, these findings clearly demonstrate a crucial role of NR2F family members, particularly NR2F2, in multiple different aspects of metabolic regulation in mice and humans. A similar finding was observed in one study in which the feed efficiency in beef cattle was studied. They identified NR2F6 as a key regulator of the hepatic inflammatory response, one of the main processes associated with feed efficiency [93].

Regarding the functional importance of the NR2F family, the focus thus far has been mainly on NR2F1 and NR2F2. However, knowledge regarding the role of NR2F1 and NR2F2 can serve as an important indicator of the functionality of the third NR2F family member, NR2F6, which has not been studied as intensively as the other family members. Based on our published [98–102] and unpublished data, NR2F6 appears to be important in cancer immunology and T-cell functionality and needs to be investigated in more detail.

2.6 NR2F6 Function in Immune Cells

The functional importance of NR2F6 has been investigated in various immune cells. In mouse macrophages, NR2F6 acts as a transcriptional repressor of cytokines, whereas in human macrophages it acts as a transcriptional activator of chemokines [103]. Formerly introduced solely as a Th17 transcriptional repressor, the role attributed to NR2F6 has gradually broadened over the years and been extended to most known immune cell types (Table 4, Ref. [98,100,103–106]). The influence of NR2F6 is particularly prominent among T-cell subsets, as NR2F6 regulates the differentiation and function of multiple subsets of CD4 and CD8 T cells, lending it a crucial position in adaptive immunity and anti-tumoral responsiveness. Examination of NR2F6 function in the germinal center response has revealed that *Nr2f6* deficiency increases the accumulation of germinal center B cells, plasma cells, and follicular T helper cells in mice that were immunized. Mechanistically, NR2F6 governs the expression of IL-21 through direct binding at several defined sites within the *Il21* locus [104].

Investigating NR2F6 functionality in T cells, our team and others have shown that NR2F6 plays an important role in autoimmunity and immune functions during cancer surveillance [98,105,107]. Our team observed that loss of *Nr2f6* exacerbates experimental autoimmune encephalomyelitis (EAE). Mechanistically, NR2F6 appears to act as a negative regulator of *Il17* transcription via direct DNA binding to the *Il17* promoter region. This abrogates NFAT/AP-1 transcription factor binding to the *Il17* gene locus, leading to robust NR2F6-mediated transrepression, thereby acting as a safe guard against EAE disease progression [105,106].

On the other hand, NR2F6 appears to be an essential signaling intermediate governing the amplitude of host-protective cancer immunity. The observation that immune cells play a role in preventing cancer cell progression was first described by Burnet and Thomas more than 60 years ago [108]. In accordance with this observation, an increased number of tumor-infiltrating T lymphocytes (TILs) is associated with better survival prognosis [109]. In several induced and spontaneous mouse tumor models, we observed that Nr2f6-deficient mice exhibit a reduction in cancer growth due to increased CD4 and CD8 T-cell tumor infiltration and augmented effector T-cell functions, such as IL-2 and IFNγ secretion at the solid tumor site. In addition, in all of these pre-clinical mouse tumor models, NR2F6 deficiency resulted in a significant survival benefit and implementation of a robust anti-tumor response against solid tumors, as well as metastases [98,100]. In heterozygous *Nr2f6*$^{+/-}$ mice, a similar benefit was observed in terms of tumor growth suppression, suggesting haploinsufficiency [100]. The relevance of NR2F6 function in human T cells was investigated by employing human NR2F6 knockdown T-cell cultures. Analysis of TILs from human NSCLC biopsies showed massive upregulation of NR2F6 at the tumor site in approximately 50% of NSCLC patients, which has been considered to produce exhausted TILs that are incapable of accomplishing sufficient immune responses against cancer [100].

Mechanistically, during T-cell activation, regulatory phosphorylation of the NR2F6 DBD serves as an important feedback mechanism. High-affinity antigen receptor signaling (i.e., PKCθ-mediated phosphorylation of Ser-83 within the DBD of NR2F6) negates the DNA-binding ability of NR2F6, enabling unopposed DNA binding of NFAT/AP-1 transcription factors at the cytokine gene loci of key effector cytokines IL-2, IFNγ, and TNFα. Thus, T-cell-intrinsic NR2F6 directly suppresses the DNA binding abilities of the transcription factors NFAT and AP-1 at defined gene loci, resulting in transrepression of, for example, the IL-2 and IFNγ transcriptional responses [105].

A schematic overview of NR2F family member functions, as discussed in this review and summarized per topic in Tables 1,2,3,4, is illustrated in Fig. 1.

3. Conclusions

Profound biochemical advances have been made in understanding how NR2F family members regulate gene expression. NR2F family members are central for cell growth and developmental processes (Fig. 1). Currently under investigation at the molecular level, one major challenge is the functional connections between NR2F homo- or hetero-dimeric complexes bound to a particular promoter, their tissue-specific co-activators and co-repressors, and the specific properties of each complex with respect to gene regulation.

Yet, the NR2F family has substantial translational importance, and only the complementary information listed below will give us a better understanding of the functions of NR2F isoforms in key mechanisms of cell-type speciation. The critical details that need to be considered are (i) genome-scale validation of NR2F isoform-specific versus NR2F family-overlapping DNA-binding specificites that control gene transcription, (ii) the signaling pathways responsible for induction of NR2F gene expression as feed-forward and/or feedback processes, (iii) epigenetic regulation by DNA methylation present in NR2F family gene promoters, and (iv) the identification of signaling pathways that respond to specific cellular stimuli for post-translational regulation, such as phosphorylation and sumoylation on NR2F protein functions.

Due to their excellent druggability, NRs are promising candidates as novel therapeutic targets for cancer therapy, and numerous clinical or preclinical trials are currently underway to assess the therapeutic efficacy of estrogen receptor and androgen receptor inhibitors [2]. NR2F6 has no known ligands thus far, but recent publications describe both closely related NR2F family members NR2F1 [71] and NR2F2 [110] as druggable targets, suggesting the possibility of finding a small-molecule antagonist that targets the immune checkpoint NR2F6 due to the shared LBD between all three NR2F family members [3]. A detailed review discussing NR2F6 as a next-generation target for cancer immunotherapy was published recently [102].

Future studies promise insights into the specific roles of NR2F isoforms in defined tissues and will ultimately not only shed light on normal NR2F protein functions, but probably also lead to unique insights into human disorders, such as cancer and autoimmunity, paving the way for novel treatment options.

Abbreviations

AP-1, activator protein 1; BBSOAS, Bosch-Boonstra-Schaaf optic atrophy syndrome; BRG-1, Brahma-related gene 1; COUP-TF, chicken ovalbumin upstream promoter transcription factor; DBD, DNA binding domain; Dll-4, delta-like protein 4; EAE, experimental autoimmune encephalomyelitis; EPC, endothelial progenitor cell; FOXO1, Forkhead box protein O1; Glut4, glucose transporter type 4; HCC, hepatocellular carcinoma; Hey2, Hes-related

family BHLH transcription factor with YRPW motif 2; HIFα, hypoxia-inducible factor alpha; HNSCC, head and neck squamous cell carcinoma; LBD, ligand binding domain; MLL-AF4, mixed-lineage leukemia acute lymphocytic leukemia 1-fused gene from chromosome 4; NFAT, nuclear factor of activated T cells; NR2F1, nuclear receptor subfamily 2 group F member 1 (synonyms: COUP-TFI, EAR-3); NR2F2, nuclear receptor subfamily 2 group F member 2 (synonyms: COUP-TFII, ARP-1); NR2F6, nuclear receptor subfamily 2 group F member 6 (synonyms: COUP-TFIII, EAR-2); NRs, nuclear receptors; NSCLC, non-small cell lung cancer; PPARγ, peroxisome proliferator-activated receptor gamma; Prox1, Prospero homebox protein 1; PTEN, phosphate and tensin homologue; RORC, retinoic acid receptor-related orphan receptor C; ROS, reactive oxygen species; SMAD4, mothers against decapentaplegic homolog 4; Sox18, SRY-box transcription factor 18; TILs, tumor-infiltrating T lymphocytes; VEGF, vascular endothelial growth factor.

Author Contributions

Conceptualization, writing and editing by TS and GB. All authors have read and agreed to the published version of the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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