Role of COUP-TFII in cardiovascular diseases and colorectal cancer: Insights into the molecular mechanisms and clinical relevance (Review)

TAE-HO PARK[1], SANG-HEUM HAN[2,3], JUN-GI CHO[2,3], SU-JEONG PARK[2,3], JIN-YEONG HAN[4] and JOO-IN PARK[2,3]

[1]Department of Internal Medicine, Dong-A University College of Medicine, Busan 49201, Republic of Korea; [2]Department of Biochemistry, Dong-A University College of Medicine, Busan 49201, Republic of Korea; [3]Department of Translational Biomedical Sciences, Dong-A University Graduate School, Busan 49201, Republic of Korea; [4]Department of Laboratory Medicine, Dong-A University College of Medicine, Busan 49201, Republic of Korea

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Abstract. Chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII), also known as nuclear receptor subfamily 2 group F member 2, is an orphan nuclear receptor that controls various biological processes, including development, angiogenesis, metabolism and tissue homeostasis. Structurally, COUP-TFII comprises a DNA-binding domain and a ligand-binding domain, facilitating its interaction with various signaling pathways, and thereby exerting diverse biological effects. Alterations of the expression or transcriptional activity of COUP-TFII are associated with various diseases, including cardiovascular diseases (CVDs) and different types of cancer such as colorectal cancer (CRC). In the context of CVDs, COUP-TFII serves a key role in the development and function of the vascular system. Dysregulation of COUP-TFII leads to aberrant angiogenesis and vascular remodeling, contributing to the pathogenesis of various CVDs. In CRC, COUP-TFII acts as either a tumor suppressor or a tumor promoter, depending on the cellular context. The present review explores the structure and regulatory mechanisms of COUP-TFII, its functions and molecular mechanisms in CVDs and CRC, and its emerging role in linking these diseases, offering insights into potential treatments and future research directions.

Correspondence to: Professor Joo-In Park, Department of Biochemistry, Dong-A University College of Medicine, 32 Daesingongwon-ro, Seo-Gu, Busan 49201, Republic of Korea
E-mail: jipark@dau.ac.kr

Abbreviations: AF, activation function; Ang-1, angiopoietin-1; APOB, apolipoprotein B; BMP4, bone morphogenic protein 4; CCL, C-C motif chemokine ligand; ChIP, chromatin immunoprecipitation; COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; CVD, cardiovascular disease; CRC, colorectal cancer; CXCL, C-X-C motif chemokine ligand; DBD, DNA-binding domain; DCM, dilated cardiomyopathy; DIHF, diabetes-induced heart failure; DR, direct repeat; EC, endothelial cell; EMT, epithelial-to-mesenchymal transition; EndMT, endothelial-to-mesenchymal transition; ERG, ETS-related gene; ETC, electron transport chain; HDAC, histone deacetylase; HIF, hypoxia-inducible factor; Mfn, mitofusin; miR, microRNA; NRCM, neonatal rat cardiomyocyte; Opa1, optic atrophy 1; PA, palmitic acid; PAH, pulmonary arterial hypertension; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphophatase 3; PGC-1α, peroxisome proliferator activated receptor γ coactivator-1α; Pink1, PTEN-induced kinase 1; PPAR, peroxisome proliferator-activated receptor; RAS, renin-angiotensin system; ROS, reactive oxygen species; Shh, sonic hedgehog; SMRT, silencing mediator for retinoid or thyroid hormone receptor; Sod2, superoxide dismutase 2; Sp1, specificity protein 1; SRC-1, steroid receptor coactivator-1; TCF7L2, T-cell factor 7-like 2 or transcription factor 7-like 2; TFPI2, tissue factor pathway inhibitor 2; Tie-2, tyrosine kinase with immunoglobulin like and EGF like domains-2; UTR, untranslated region; aa, amino acids

Key words: COUP-TFII, CVD, CRC

Contents

1. Introduction

Chicken ovalbumin upstream promoter-transcription factor II [COUP-TFII; also known as nuclear receptor subfamily 2 group F member 2 (NR2F2)] is a member of the steroid/thyroid nuclear receptor superfamily (1,2). COUP-TFII is an orphan nuclear receptor that regulates the transcription of numerous genes in multiple physiological and pathological conditions (3), and can adjust cellular processes, such as angiogenesis, metabolism and differentiation, as a transcriptional regulator (3-5). While it can act as either a transcriptional activator or repressor, the latter appears to be more common (6).

Using conventional knockout and tissue-specific conditional knockout mice, studies have revealed that COUP-TFII serves an important role in heart development, angiogenesis and vein identity determination (7-10). Dysregulation of COUP-TFII has been implicated in the pathogenesis of both cardiovascular diseases (CVDs) and colorectal cancer (CRC) (3). In CVDs, COUP-TFII serves a role in vascular remodeling and energy metabolism (3), while in CRC, it may function as either a tumor suppressor or promoter, depending on the cellular context (3,11). However, the understanding of the detailed molecular mechanisms linking COUP-TFII to these diseases remains incomplete.

CVDs and CRC are common causes of mortality and morbidity worldwide (12). Growing evidence suggests a shared pathophysiology between CVDs and CRC, despite these being separate disease entities (13-15). In addition, CVDs and CRC have overlapping modifiable risk factors, such as diet, physical inactivity and chronic inflammation (16). The development of therapeutic modalities has improved the survival rates of patients with CRC, thus increasing the numbers of CRC survivors who are at higher risk for a first or subsequent CVD event; this is partly attributable to the shared pathophysiology of the two diseases, and the cardiotoxic effect of anticancer treatment (15). It is reasonable to hypothesize that therapeutic strategies to improve modifiable CRC risk factors will also have a beneficial effect on CVD prevention and vice versa.

The present review aims to summarize the structure and regulatory mechanisms of COUP-TFII, to review its mechanistic roles in CVDs (Table I) and CRC (Table II) by focusing on published papers identified using PubMed (https://pubmed.ncbi.nlm.nih.gov/) with the key words 'COUP-TFII (NR2F2)' and 'CVDs', 'COUP-TFII (NR2F2)' and 'CRC', or 'CVDs' and 'CRC', and to provide future research directions.

Literature search. A comprehensive literature search was conducted using PubMed (https://pubmed.ncbi.nlm.nih.gov/) to identify relevant studies on COUP-TFII in CVDs or in CRC. The search covered the literature from January 1900 to January 2025. The inclusion criteria were: i) Original research articles and reviews investigating COUP-TFII expression and function in CVDs or CRC; ii) studies using human tissue samples, cell lines or animal models; iii) original articles and reviews under the key words 'CVDs' and 'CRC'; and iv) articles published in English. The exclusion criteria were: i) Studies lacking specific data on COUP-TFII; and ii) non-English publications. All authors screened the articles, and any discrepancies were resolved through discussion.

2. Structure and regulatory mechanisms of COUP-TFII

Structural characteristics. As shown in Fig. 1A, COUP-TFII is composed of six regions containing three main domains: An N-terminal domain containing a transcriptional activation function (AF) motif [1-78 amino acids (aa)], a DNA-binding domain (DBD; 79-151 aa) and a C-terminal ligand binding domain (LBD; 177-411 aa), separated by a hinge region (D) (2,3,17).

The N-terminal A/B region containing AF1 motif is required for the recruitment of coactivators or corepressors. DBD, located in the C region, contains two zinc-finger motifs that enable COUP-TFII to bind to specific DNA sequences referred to as direct repeat (DR) motifs composed of the AGGTCA sequence. These sequences are commonly found in the regulatory regions of target genes involved in developmental and metabolic pathways. To regulate a variety of target genes, the DBD of COUP-TFII recognizes and binds to DR elements and palindromic sequences with variable spacing (DR-1, DR-2 and DR-4) within the promoter regions of target genes (2,3,17).

The LBD, located in the E region, facilitates interactions with coactivators such as steroid receptor coactivator-1 (SRC-1) and peroxisome proliferator activated receptor γ coactivator-1α (PGC-1α), and corepressors such as nuclear receptor corepressor (NCoR) and silencing mediator for retinoid or thyroid hormone receptor (SMRT) (2,3,17).

Although COUP-TFII lacks a known endogenous ligand, the LBD modulates transcription through allosteric interactions with coactivators or corepressors. Various classes of molecules can interact with the LBD of COUP-TFII, including endogenous lipids and metabolites (such as retinoic acid derivatives, fatty acids and phospholipids), synthetic small molecules, steroid hormones, peptides and coregulatory proteins (such as NCoR, SMRT and SRC-1). The AF2 motif, located within the LBD, is pivotal for the recruitment of coactivators or corepressors and modulating transcriptional activity. The F region refers to the C-terminal region of the COUP-TFII protein (2,3,17).

COUP-TFII is a ligand-regulated nuclear receptor. Kruse *et al* (18) demonstrated that COUP-TFII adopts an auto-repressed conformation, as revealed by crystallographic analysis, due to the interaction between the co-factor binding sites and the AF2 motif, preventing co-factor recruitment in the absence of ligands. Ligands, such as retinoic acids, can activate COUP-TFII by releasing it from its auto-repressed conformation (18). The findings of this integrative approach can enhance the translational value of the structural analyses of COUP-TFII in the absence or presence of ligands in drug development. However, to the best of our knowledge, the physiological relevance of ligand regulation remains unclear, with further studies being required to identify endogenous ligands and validate tissue-specific effects.

COUP-TFII can function as either a negative or positive transcriptional regulator via direct binding to DNA response elements (DR sites) or interaction with other transcription factors [such as retinoid x receptor and specificity protein 1 (Sp1)], respectively (3,17). COUP-TFII can inhibit the transcription of target genes via the recruitment of corepressors through the formation of homodimers or heterodimers (Fig. 1B) (2,3). Alternatively, it can also bind to Sp1 sites to

Table I. Roles and molecular mechanisms of COUP-TFII in the vasculature and heart.

First author/s, year	Functions of COUP-TFII	Molecular mechanisms	Pathological implications	(Refs.)
Pereira *et al*, 1999; Wu *et al*, 2013; You *et al*, 2005; Chen *et al*, 2012; Al Turki *et al*, 2014; Sissaoui *et al*, 2020	Vascular development and patterning; expressed in venous ECs, atria, coronary arteries, aorta and VSMCs; acts as a molecular switch between venous and arterial differentiation	Binds to cis-regulatory elements (e.g., *HEY2* and *FOXC1*) to repress arterial markers; recruits HDACs in venous ECs to actively repress *HEY2*; interacts with *ERG* to promote vein-specific gene expression; the -161K enhancer functions as a bimodal switch based on COUP-TFII occupancy	Loss or forced expression disrupts normal vascular identity, leading to aberrant arterial marker expression in veins and vice versa	(7,8,10, 42,44,45)
Cui *et al*, 2015	Adult vascular endothelium maintenance-maintains venous identity	Direct binding to the *BMP4* promoter to repress its expression; regulates inflammatory (e.g., *CXCL10*/*11* and *CCL5*) and antithrombotic (for example, *TFPI2*) genes to prevent EndMT	Dysregulation can trigger EndMT, inflammation and altered antithrombotic responses	(46)
Qin *et al*, 2010	Angiogenesis - promotes vessel stability and maturation	Directly upregulates Ang-1 expression by binding with Sp1 to the Ang-1 promoter	Supports the formation of stable blood vessels under physiological conditions; disruption may impair vessel maturation	(20)
Dougherty *et al*, 2023; Cao *et al*, 2019; Talati and Hemnes, 2015; Poels *et al*, 2020; Rodríguez-García *et al*, 2017	Pathological angiogenesis and metabolic dysregulation; involvement in atherosclerosis and PAH	Knockdown of COUP-TFII activates AKT/STAT signaling and increases DKK1 expression; upregulates glycolytic genes (e.g., *HK2*, *LDHA* and *PFKFB3*), enhancing oxidative stress and lactate production	Elevated glycolysis and lipogenesis destabilize atherosclerotic plaques and promote endothelial proliferation, contributing to PAH pathogenesis; overall, dysregulation leads to a pro-inflammatory, hyperproliferative and migratory endothelial phenotype	(56-60)
Periera *et al*, 1999; Lin *et al*, 2012; Al Turki *et al*, 2014; Nakamura *et al*, 2011; Qiao *et al*, 2018; Upadia *et al*, 2018; Cornea *et al*, 2008; Botto *et al*, 2001; Perilhou *et al*, 2008; Vilhais-Neto *et al*, 2010; Gruber and Epstein, 2004	Heart development-essential for proper cardiac morphogenesis; conventional knockout leads to embryonic lethality (embryonic day 10) with poorly developed atria, cardinal veins and hemorrhagic vessels; hypomorphic mutants display atrioventricular septal, valvular defects and abnormal coronary morphogenesis	Regulates transcriptional activation (missense variants affect activator function while repressor function is preserved); interacts with dosage-sensitive transcription factors (GATA4, TBX5 and NKX2-5); environmentally responsive (modulated by high glucose and retinoic acid via pathways such as the Foxo1 pathway)	Congenital heart defects (atrial/ ventricular septal defects, aortic stenosis, coarctation of the aorta, DORV and VSD)	(7,9,44, 61-68)

Table I. Continued.

First author/s, year	Functions of COUP-TFII	Molecular mechanisms	Pathological implications	(Refs.)
Wu *et al*, 2015; Kittleson *et al*, 2005; Hannenhalli *et al*, 2006	Adult heart - mitochondrial and metabolic regulation; maintains mitochondrial function and metabolic homeostasis in cardiomyocytes	Overexpression causes defects in the ETC; downregulates genes for mitochondrial fusion and quality control (Mfn1, Mfn2, Opa1 and Pink1); suppresses the PGC-1α-Sod2 pathway, leading to the accumulation of damaged mitochondria; alters fatty acid and glucose metabolism	Mitochondrial dysfunction and oxidative stress, leading to heart failure; development of metabolic inflexibility in heart failure	(69-71)
Wu *et al*, 2015; Miao *et al*, 2022; Tan *et al*, 2020; Dhalla *et al*, 2020; Dixon *et al*, 2012; Li *et al*, 2020 Ma *et al*, 2020; Tang *et al*, 2021; Wang *et al*, 2022	Elevated COUP-TFII expression is observed in DIHF	Promotes ferroptosis (an iron-dependent, non-apoptotic cell death marked by lipid ROS accumulation) and mitochondrial dysfunction; knockdown reduces mitochondrial damage and ferroptosis in palmitic acid-treated neonatal rat cardiomyocytes; direct regulation of mitochondrial metabolic regulator genes (for example, PPARα and PGC-1α)	Exacerbation of diabetic heart failure via increased oxidative stress, mitochondrial damage and ferroptosis	(69,72-79)

COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; EC, endothelial cell; VSMC, vascular smooth muscle cell; HDAC, histone deacetylase; Hey2, hairy and enhancer of split-related; ERG, ETS related gene; TFPI2, tissue factor pathway inhibitor 2; BMP4, bone morphogenic protein 4; CXCL10/11, C-X-C motif chemokine ligand 10/11; CCL5, C-C motif chemokine ligand 5; EndMT, endothelial-to-mesenchymal transition; Sp1, specificity protein 1; Ang-1, angiopoietin-1; PAH, pulmonary arterial hypertension; DKK1, Dickkopf-related protein 1; HK2, hexokinase 2; LDHA, lactate dehydrogenase A; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphophatase 3; DORV, double outlet of right ventricle; VSD, ventricular septal defect; ETC, electron transport chain; Mfn, mitofusin; Opa1, optic atrophy 1; Pink1, PTEN-induced kinase 1; PGC-1α, peroxisome proliferator activated receptor γ coactivator 1α; Sod2, superoxide dismutase 2; DIHF, diabetes-induced heart failure; ROS, reactive oxygen species; PPARα, peroxisome proliferator activated receptor α; TBX5, T-box transcription factor 5; GATA4, GATA binding protein 4; NKX2-5, NK2 homeobox 5.

activate the transcription of target genes [such as neuropilin 2 and angiopoietin-1 (Ang-1); Fig. 1B] (19,20). The experimental evidence for this mechanism was mainly obtained in *in vitro* studies and tumor models (19,20). Qin *et al* (20) used a conditional *COUP-TFII* knockout strategy in xenograft models to demonstrate that COUP-TFII positively regulated tumor angiogenesis by directly binding to Sp1 binding sites in the promoter regions of angiogenesis-related genes. These findings were supported by robust promoter-reporter assays, chromatin immunoprecipitation (ChIP) and loss-of-function experiments, providing convincing mechanistic evidence. Therefore, in cancer biology contexts, the experimental quality of these studies (19,20) is high. However, beyond oncogenic models, their physiological relevance has yet to be validated. Further studies using cardiovascular- or angiogenesis-specific models are necessary to confirm the functional relevance of COUP-TFII-Sp1 interactions in non-cancer conditions.

Regulatory mechanisms of COUP-TFII expression and activity. The regulatory mechanisms of COUP-TFII expression and activity are not completely understood, because its specific ligand has not yet been fully characterized. The expression and activity of COUP-TFII are tightly regulated by various mechanisms, such as transcriptional and epigenetic regulation, and by interactions with signaling pathways.

3. Transcriptional regulation of COUP-TFII expression

Upstream regulators of COUP-TFII expression. COUP-TFII expression has been reported to be modulated by sonic hedgehog (Shh) during developmental processes, because an Shh-responsive element was identified in the COUP-TFII promoter (21). This finding was based on *in vitro* promoter assays, and although revealing a potential regulatory element, *in vivo* results were lacking. Thus, the physiological significance of this regulation has yet to be further validated. COUP-TFII expression has also been reported to be regulated by retinoic acid (22,23), estradiol (24) or MAPK pathways (25). Retinoic acid and its derivatives regulate COUP-TFII expression via nuclear receptor-mediated pathways. This regulation

Table II. Comparative summary of COUP-TFII functions in CRC in different molecular contexts.

First author/s, year	Context/condition	Role of COUP-TFII	Molecular mechanisms/marker	(Refs.)
Shin *et al*, 2009; Yun *et al*, 2017	High expression levels in tissue microarray (early studies)	Tumor suppressor	Associated with improved survival, ↓ LN metastasis	(80,81)
Yun and Park, 2020	Overexpression in SNU-C4 cells	Tumor suppressor	↑ p53, ↑ PTEN, ↓ Akt signaling	(82)
Yun *et al*, 2020	Knockdown in HT-29 cells	Tumor suppressor	↑ Akt → ↓ GSK-3β → ↑ β-catenin, ↑ MMP7, ↑ FOXC1	(83)
Bau *et al*, 2014; Cano *et al*, 2000; Li *et al*, 2007; Kudo- Saito *et al*, 2009	High expression levels in EMT-prone CRC cells	Tumor promoter	Directly activates Snail1 transcription → EMT (by downregulating E-cadherin and enhancing immunosuppression)	(84,86-88)
Wang *et al*, 2017	miR-21 overexpression	Tumor promoter	↑ COUP-TFII → inhibits Smad7 → TGF- β-induced EMT	(90)
Bao *et al*, 2019	COUP-TFII regulation of miR-34a	Tumor promoter	COUP-TFII → ↓ tumor-suppressor miR-34a → ↑ cell migration, ↓ chemosensitivity	(91)

COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; CRC, colorectal cancer; LN, lymph node; EMT, epithelial-to-mesenchymal transition; miR, microRNA.

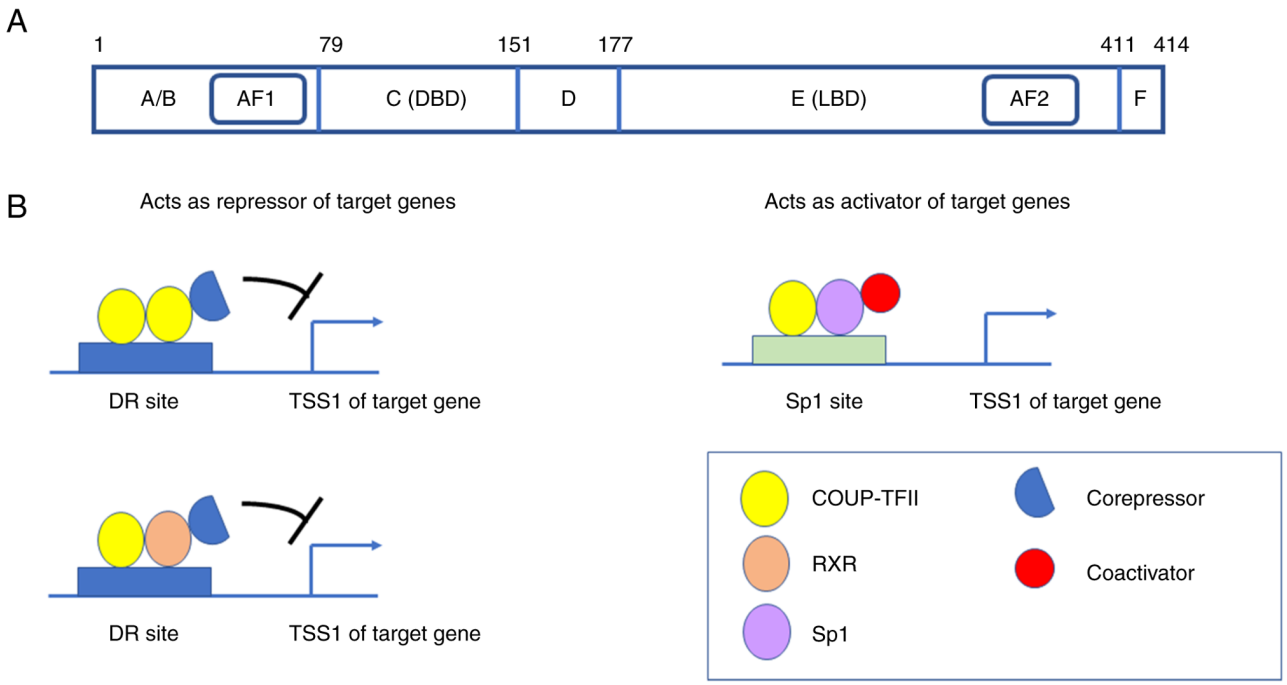

Figure 1. Structure and transcriptional regulatory mechanism of COUP-TFII. (A) Schematic structure of the human COUP-TFII protein. The numbers represent the positions of amino acids. (B) Transcriptional regulatory mechanism of COUP-TFII. COUP-TFII binds to the 5'-AGGTCA-3' motif or palindromic sequences with various spacings (DR site), either directly (homodimer) or indirectly, through heterodimer formation with other proteins (such as RXR) to regulate downstream target gene expression. COUP-TFII can also bind to Sp1 sites via interaction with Sp1 to cooperatively activate gene expression. COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; RXR, retinoid X receptor; Sp1, specificity protein 1; TSS1, transcription start site 1; DBD, DNA-binding domain; LBD, ligand-binding domain; AF, activation function; DR, direct repeat.

is important to maintain tissue homeostasis and differentiation (22,23). However, since the study by Soosaar *et al* (23) was solely based on *in vitro* experiments using a combination of cell culture and biochemical assays, their findings may not be generalized. In addition, retinoic acid has been reported to suppress COUP-TFII expression in some contexts (such as the rat glioblastoma C3 and human glioblastoma U373 cell lines), suggesting that the cellular context and specificity of receptor isoforms influence the outcome of retinoic acid signaling (23). COUP-TFII expression is also regulated by other factors,

including Oct4 (26), microRNA (miRNA/miR)-302 (26) and Ets-1 (27). COUP-TFII expression has been revealed to be repressed by Oct4, which binds to the COUP-TFII promoter, and by miR-302, which interacts with its 3'-untranslated region (3'-UTR) (26). Oct4, as a key pluripotency factor, interacts with COUP-TFII to regulate its transcriptional activity, particularly in stem cell maintenance and differentiation (26). The findings of Rosa and Brivanlou (26) were based on overexpression and knockdown experiments without addressing potential off-target effects; however, in stem cell models, the results were comprehensive. Furthermore, the suppressive effect of Oct4 on COUP-TFII may not be conserved in non-pluripotent cell types, suggesting that this regulation may be limited by tissue specificity (26). Petit *et al* (27) revealed that Ets-1, together with SRC-1, could activate COUP-TFII expression in luciferase reporter assays. This study is insightful for providing explainable potential mechanisms. However, its conclusions are derived from *in vitro* assays, and direct binding of Ets-1 to the endogenous promoter in physiological conditions has yet to be fully demonstrated. COUP-TFII expression is repressed by hypoxia-inducible factor (HIF)-1α in hypoxic endometrial stromal cells (28) and by the artery-specific Δ-like canonical Notch ligand 4 - Notch-hairy/enhancer of split-related with YRPW motif protein 2 (Hey2) signaling axis in endothelial progenitor cells under hypoxic conditions (29). Both studies (28,29) emphasized the importance of hypoxic signaling; however, since the results are confined to specific cell types, they do not address whether similar regulation occurs in other cell lineages.

miRNAs are known to be important post-transcriptional regulators in cancer (30). In particular, Yun and Park (11) reviewed regulation of COUP-TFII expression by miRNAs. For example, miRNA-27b downregulates COUP-TFII expression through interaction with its 3'-UTR, leading to the inhibition of proliferation and invasion of gastric cancer cells *in vitro*, and metastasis of gastric cancer cells to the liver *in vivo* (31). A study by Feng *et al* (31) demonstrated both *in vitro* and *in vivo* effects, which reinforces its conclusions. However, the mechanistic association between COUP-TFII suppression and phenotypic outcomes was not directly validated by rescue experiments, leaving room for alternative explanations. Additionally, miR-302, miR-302a, miR-382, miR-101, miR-27a and miR-194 have been reported to downregulate COUP-TFII expression, and to have diverse functions in different cell types, such as embryonic stem cells, mesenchymal C3H10T1/2 cells, colorectal cancer cells and prostate cancer cells (26,32-37). These studies used luciferase reporter assays, reverse transcription-quantitative PCR and western blot analyses to demonstrate miRNA-mediated repression of COUP-TFII. However, the studies have several limitations. For example, Rosa and Brivanlou (26) based their findings on embryonic stem cells, limiting their applicability to cancer. For miR-382 and miR-101 (34-36), COUP-TFII downregulation was observed, but indirect effects could not be excluded. In the case of miR-27a (36), opposing roles in different tissues suggest context dependence. The study by Jeong *et al* (37) demonstrated that miR-194 functions as a key regulator of COUP-TFII in mesenchymal stem cells, directing their fate toward differentiation into osteoblasts and adipocytes.

Overall, although evidence supports the miRNA-based regulation of COUP-TFII, context specificity, functional redundancy and cell type-dependent expression complicate the identification of consistent regulatory mechanisms.

4. Epigenetic regulation of COUP-TFII expression

Methylation of DNA, usually at CpG island sites in promoters of genes, has been reported to inhibit transcription by interfering with the binding of transcription factors (38,39). Using the CpGPLOT program from the European Bioinformatics Institute website (http://www.ebi.ac.uk/emboss/cpgplot), Al-Rayyan *et al* (40) revealed that the CpG island is located in the promoter and within exon 1 of COUP-TFII, and demonstrated that transcription of COUP-TFII might be regulated by DNA methylation. The authors also revealed that methylation of the COUP-TFII promoter led to its reduced expression, which contributed to resistance to antiestrogen treatment in endocrine-resistant breast cancer cells (40). This study provides important evidence associating epigenetic repression of COUP-TFII with therapeutic resistance, using bioinformatics prediction (CpGPLOT), bisulfite sequencing and expression analysis. However, the findings were based on a specific endocrine-resistant breast cancer cell line (MCF-7-derived), which limits the generalizability. Furthermore, while COUP-TFII repression was associated with resistance, functional rescue assays were not performed, and thus, the causal relationship is undetermined.

A total of four isoforms (Iso1, Iso2, Iso3 and Iso4) are encoded by the *NR2F2* gene. NR2F2-Iso1 is a full-length protein composed of an N-terminal AF1 motif, a DBD, a hinge region and an LBD containing a ligand-dependent AF2 motif. NR2F2-Iso2, NR2F2-Iso3 and NR2F2-Iso4 are different from NR2F2-Iso1 in their N-terminal sequences and do not have a DBD (41). Davalos *et al* (41) demonstrated that when neural crest cells (NCCs) differentiated into melanocytes, NR2F2-Iso2 expression was silenced by DNA methylation, whereas during the progression of metastatic melanoma, this process was reversed. Thus, the study suggested that DNA methylation and demethylation serve important roles in the progression of metastatic melanoma, and their regulation of NR2F2 activity through NR2F2-Iso2 contributes to the acquisition of NCC-like and epithelial-to-mesenchymal transition (EMT)-like features in transformed melanocytes (41).

Davalos *et al* (41) combined genome-wide methylation profiling with functional assays to reveal that epigenetic reactivation of NR2F2-Iso2 promoted melanoma progression. While the study provided supportive evidence, it depended on melanoma cell lines and xenografts, which may not fully reflect the heterogeneity of human tumors. Additionally, since the association between NR2F2-Iso2 and EMT-like features was based on correlative data and functional assays, the direct molecular targets remain unknown.

The role of NR2F2 isoforms appears to be context-dependent. In contrast to their oncogenic role in melanoma, some truncated isoforms lacking the DBD exhibit growth-suppressive effects in breast and lung cancer. These discrepancies suggest that isoform function is influenced by cell type, co-regulators and signaling context. Therefore, the pro-metastatic role of NR2F2-Iso2 should not be generalized

across all types of cancer. Further research should be performed to define isoform-specific mechanisms and interactions in diverse tumor types.

5. Interactions with signaling pathways

MAPK pathway. Activation of the MAPK pathway has been reported to increase COUP-TFII expression in certain cellular contexts. For example, in breast cancer cell lines with elevated MAPK activity, COUP-TFII levels are increased (25), suggesting that this pathway can upregulate COUP-TFII as part of a broader cellular response to growth signals. However, the precise relationship between MAPK signaling and COUP-TFII expression is complex, and appears to vary depending on the specific cell type and environmental context. This study examined the association between MAPK signaling and COUP-TFII upregulation in breast cancer models using pharmacological inhibitors, suggesting an association between pathway activation and COUP-TFII expression (25). However, it lacks evidence regarding direct transcriptional regulation by MAPK effectors, such as ERK, ETS like-1 protein or c-fos. The study also used a limited number of cell lines, and failed to investigate potential pathway interactions, such as crosstalk with PI3K/AKT or hormonal signaling.

Notch signaling. Notch signaling serves an antagonistic role in regulating COUP-TFII expression (10,29). In endothelial cells (ECs), activation of Notch signaling leads to a reduction in COUP-TFII levels, which in turn promotes arterial differentiation (10,42). Conversely, COUP-TFII can inhibit Notch signaling to maintain venous identity, thereby influencing cell fate decisions during vascular development (10,42). This reciprocal regulation emphasizes the role of COUP-TFII in establishing and preserving vascular heterogeneity. Taken together, these findings are supported by studies (10,42) using genetic mouse models, lineage tracing and molecular assays that demonstrate a causal association between Notch activation and COUP-TFII downregulation in ECs during vascular development. However, the majority of the studies (10,42) focused on embryonic or early postnatal stages, leaving its role in adult or pathological conditions unclear. It is also uncertain whether this regulatory axis applies to other vascular beds, such as lymphatic or tumor endothelium. While mutual antagonism between COUP-TFII and Notch in development processes has been established, its dynamics in disease contexts require further investigation across diverse tissues.

Wnt/β-catenin pathway. COUP-TFII expression has been reported to be upregulated by the Wnt/β-catenin pathway (43). ChIP assays have demonstrated that β-catenin/T-cell factor 7-like 2 or transcription factor 7-like 2 (TCF7L2) complexes bind directly to the COUP-TFII promoter, leading to increased transcription. This interaction is notable in the context of cell differentiation. For example, activation of Wnt signaling, leading to the upregulation of COUP-TFII via β-catenin/TCF7L2 complexes, results in the suppression of adipocyte differentiation by inhibiting the expression of the adipogenic transcription factor peroxisome proliferator-activated receptor (PPAR)γ (43). Okamura *et al* (43) demonstrated that β-catenin/TCF7L2 directly bound to the COUP-TFII promoter in ChIP and reporter assays in preadipocyte models, and confirmed Wnt-mediated regulation through gain- and loss-of-function approaches. However, the focus of the study on adipocytes limited the generalizability to other cell types. It also remains undefined whether upregulation of COUP-TFII is a primary Wnt response, or a downstream effect. Broader *in vivo* and multi-lineage studies are needed to fully define the mechanisms by which Wnt signaling modulates COUP-TFII expression.

6. COUP-TFII in CVDs

Roles and molecular mechanisms of COUP-TFII in the vasculature: From developmental patterning to pathological angiogenesis. COUP-TFII is predominantly expressed in venous ECs, the atria, coronary arteries, the aorta (44) and vascular smooth muscle (8). COUP-TFII is important for proper vascular development (7). An *in vivo* study revealed that genetic ablation of COUP-TFII induces the ectopic expression of arterial markers in venous ECs, whereas forced expression of COUP-TFII in arterial cells suppresses arterial gene expression, underscoring its role as a molecular switch in vascular differentiation (10). Mechanistically, COUP-TFII binds to cis-regulatory elements at key loci, such as *HEY2* and *FOXC1* which are required for arterial differentiation. By binding to their regulatory regions, COUP-TFII inhibits the activation of the arterial differentiation program (42). A study by Sissaoui *et al* (45) demonstrated that in venous ECs, COUP-TFII recruited histone deacetylases (HDACs) to actively repress the expression of *HEY2*, a key mediator of arterial identity. Furthermore, COUP-TFII interacted with ETS-related gene (*ERG*) to induce the expression of several vein-specific genes, such as family with sequence similarity 174 member B, a disintergrin and metalloprotease with thrombospondin motifs 18 and LIM homeobox 6 (45).

The working model proposed by Sissaoui *et al* (45) suggests that a regulatory element (the -161K enhancer) acts as a bimodal switch depending on the cellular context: In venous ECs, COUP-TFII occupies this enhancer to block ERG-mediated activation of *HEY2*, whereas in arterial ECs lacking COUP-TFII, ERG binds to the -161K enhancer and promotes arterial gene expression. In the study by Sissaoui *et al* (45), CRISPR-CRISPR-associated protein 9 enhancer deletion, ChIP-sequencing and reporter assays were effectively used to define the COUP-TFII-HDAC-ERG axis and demonstrate the role of COUP-TFII as a repressor of arterial identity in venous ECs. However, the focus on embryonic tissues and human umbilical vein ECs limits the relevance to adult vasculature and pathological angiogenesis. In pathological settings such as tumors, COUP-TFII expression can persist in vessels with arterial features, suggesting that its regulatory role may be more flexible than previously suggested (20). Broader, context-specific studies are needed to clarify its function in vascular remodeling.

Cui *et al* (46) revealed the role and molecular mechanism of COUP-TFII in adult vascular endothelium, demonstrating that knockdown of COUP-TFII in adult vascular ECs upregulated the expression levels of inflammatory genes [such as C-X-C motif chemokine ligand (CXCL)10, CXCL11 and C-C motif chemokine ligand (CCL)5], while downregulating

the expression levels of antithrombotic genes [such as tissue factor pathway inhibitor 2 (TFPI2)]. This dysregulation could trigger endothelial-to-mesenchymal transition (EndMT). Furthermore, the expression levels of arterial markers (such as ephrin-B2 and hes family bHLH transcription factor 1/4), expression of genes involved in angiogenesis [such as endothelial PAS domain protein 1/HIF-2α, ephrin-A1, tyrosine kinase with immunoglobulin like and EGF like domains-2 (Tie-2) and ephrin-A4] and the osteogenic potential leading to calcium deposition of adult ECs were suppressed by COUP-TFII overexpression. Cui *et al* (46) demonstrated that the actions of COUP-TFII in adult ECs may be mediated by its negative regulation of bone morphogenic protein 4 (BMP4) through direct binding to the BMP4 promoter. These results suggest that COUP-TFII serves a key role in maintaining the venous identity in adult ECs, as well as during development (46). This study focused on adult ECs, a less explored context for COUP-TFII. Cui *et al* (46) used *in vitro* knockdown/overexpression and ChIP assays to reveal that COUP-TFII suppressed pro-inflammatory, angiogenic and osteogenic gene programs, reinforcing their conclusions. However, reliance on cultured ECs limits *in vivo* relevance, while other pathways regulating BMP4 were not fully excluded. Their findings suggest anti-inflammatory and anti-osteogenic roles of COUP-TFII in adult ECs, which are inconsistent with reports of its pro-angiogenic and immunosuppressive effects in tumor models (17,20). These discrepancies underscore context-dependent functions of COUP-TFII and emphasize the need for further investigations in normal vs. diseased adult vasculature.

Angiogenesis, the formation of new blood vessels, relies on coordinated interactions among shear stress, vascular growth factors such as VEGF and Ang-1 (a ligand for Tie-2), intracellular signaling pathways (such as Notch) and intercellular contacts (47-54).

Knockdown experiments in mouse aortic vascular smooth muscle cells and C3H10T1/2 fibroblasts have revealed that COUP-TFII positively regulates Ang-1 expression levels (20). This regulation occurs through the direct binding of COUP-TFII, in cooperation with Sp1, to the Ang-1 promoter. The upregulation of Ang-1 by COUP-TFII suggests a pro-angiogenic role, promoting vessel stability and maturation (20). However, this study (20) was limited by its dependence on *in vitro* fibroblast and smooth muscle cell models, which may not reflect the *in vivo* angiogenic environment. Although ChIP assays verified COUP-TFII binding to the Ang-1 promoter, its functional relevance in ECs *in vivo* remains unvalidated. Furthermore, the role of COUP-TFII in angiogenesis appears to be context-dependent, while it can promote Ang-1 expression, another study has revealed that it may suppress VEGF receptor expression and inhibit sprouting, particularly in tumors (55). These conflicting findings suggest that COUP-TFII may balance vascular stabilization and angiogenic modulation in a tissue- and context-specific manner.

Dysregulation of COUP-TFII can lead to pathological angiogenesis, contributing to various vascular diseases, such as atherosclerosis and pulmonary arterial hypertension (PAH) (56). For instance, in multiple types of human ECs (e.g., lung microvascular, coronary artery and pulmonary artery ECs), knockdown of COUP-TFII activates the AKT and STAT signaling pathways. This activation is accompanied by an increase in Dickkopf-related protein 1 expression, resulting in a pro-inflammatory, proliferative, hypermigratory and apoptosis-resistant phenotype. These changes are further characterized by the induction of EndMT, increased oxidative stress, enhanced lactate production and the upregulation of glycolytic genes, such as hexokinase 2, lactate dehydrogenase A and 6-phosphofructo-2-kinase/fructose-2,6-bisphophatase 3 (PFKFB3) (56). The strengths of this study include the use of multiple types of ECs, and the integration of molecular, metabolic and phenotypic analyses (56). However, key limitations include the reliance on *in vitro* knockdown models, which may not fully reflect the complexity of the *in vivo* vascular environment.

Suppression of COUP-TFII has been associated with the induction of glycolytic and lipogenic pathways (57,58). Elevated PFKFB3 levels, in particular, destabilize atherosclerotic plaques and contribute to the progression of atherosclerosis (59). Similarly, enhanced endothelial glycolysis and proliferation, driven by COUP-TFII suppression, may underlie the pathogenesis of PAH (57,60). These studies are notable for their mechanistic data associating metabolic shifts with vascular dysfunction and their use of relevant EC models (57,60). However, limitations include a lack of *in vivo* causal data, and uncertainty regarding whether the metabolic changes are a primary driver, or a secondary effect, of the diseases. Furthermore, other studies suggest that COUP-TFII loss may induce protective metabolic adaptations in other contexts, indicating potential tissue- or disease-specific variability that warrants further investigation (3,5).

7. Role of COUP-TFII in the heart

Knockout mouse models and human genetic analyses have been used to elucidate the role of COUP-TFII in the heart during development (7,9,44,61-63). Conventional COUP-TFII knockout mice die at embryonic day 10, exhibiting poorly developed atria and cardinal veins with enlarged hemorrhagic vessels, while hypomorphic mutants are born with atrioventricular septal and valvular defects, as well as abnormal coronary morphogenesis (7,9). EC-specific COUP-TFII knockout mice exhibit embryonic lethality due to vascular defects, including hypoplastic endocardial cushions, hemorrhage, and thin, dilated vessels (9). This study provided *in vivo* evidence for the key role of COUP-TFII in vascular development, using a genetically controlled, cell type-specific knockout model (9). However, limitations include the early lethality of the models, which restricts analysis to embryonic stages, and the lack of mechanistic investigation of downstream pathways. To the best of our knowledge, while no direct conflicting results have been reported, some studies have suggested that the function of COUP-TFII may differ in postnatal or under pathological conditions (17,20,55), indicating potential stage-specific roles that require further research.

Human genetic studies have revealed that COUP-TFII missense variants or 15q terminal deletions encompassing COUP-TFII are associated with atrial or ventricular septal defects, either in isolation or in combination with other congenital heart defects, such as aortic stenosis and coarctation of the aorta (44,61-63). *In vitro* experimental data indicate that all six COUP-TFII missense variants (Gln75dup, Asp170Val,

Asn205Ile, Glu251Asp, Ser341Tyr and Ala412Ser), identified in patients with atrioventricular septal defects, have a measurable impact on the transcriptional activator function of COUP-TFII in at least one of two assays: Nerve growth factor-induced protein A or apolipoprotein B (APOB) promoter-driven luciferase assays in HEK293 cells. By contrast, the repressor function of COUP-TFII appears to remain intact, as revealed by APOB promoter-driven luciferase assays in HEPG2 cells. The promoter-specific effects of these individual mutations likely reflect the complexity of the protein-protein interactions involving COUP-TFII, which vary, depending on the tissue, developmental stage and genomic context (44). The strengths of this study include the integration of genetic and functional data; however, limitations derive from the use of non-cardiac, overexpression-based assays that may not accurately reflect *in vivo* cardiac transcriptional networks (44). In addition, the mutation-specific and promoter-dependent effects underscore the complexity of COUP-TFII regulation, while for the majority of variants, no *in vivo* confirmation of pathogenicity has been provided.

COUP-TFII may also function as an environmentally responsive factor by mediating the effects of known non-genetic congenital heart disease risk factors, such as high glucose (64) and retinoic acid levels (65). Insulin and glucose have been revealed to suppress COUP-TFII expression via the Foxo1 pathway in hepatocytes and pancreatic cells (66). Furthermore, COUP-TFII serves an important role in retinoic acid signaling during development (67). However, to the best of our knowledge, direct evidence for its role in the developing heart is scarce. Thus, the translational relevance of these findings remains uncertain. Further studies are required to determine how glucose and retinoic acid levels may alter COUP-TFII expression in the developing heart. Additionally, COUP-TFII has been reported to interact with other dosage-sensitive transcription factors that are key for heart formation, including GATA binding protein 4 (GATA4), T-box transcription factor 5 and NK2 homeobox 5 (68). Specifically, a novel heterozygous mutation (p.G83X) in the COUP-TFII gene was identified in a family affected by congenital double outlet right ventricle and ventricular septal defect. Functional investigations demonstrated that the COUP-TFII G83X-mutant protein exhibited no transcriptional activity. Furthermore, the mutation abrogated the synergistic transcriptional activation of COUP-TFII with GATA4 (62). These functional assays provide mechanistic insights, but are limited by the use of overexpression systems in non-cardiac cells. No *in vivo* validation or comprehensive mutational analysis was carried out, and the interaction between COUP-TFII and environmental signals in human cardiogenesis has yet to be fully elucidated.

The role and molecular mechanisms of COUP-TFII in the adult heart have been investigated using COUP-TFII overexpression mouse models (69), and two independent cohorts of patients with non-ischemic dilated cardiomyopathy (DCM) (70) and idiopathic DCM (dataset no. GSE406) (71). Using COUP-TFII-overexpressing mice and rescue experiments through the removal of COUP-TFII in calcineurin-transgenic (CnTg/Cre/F^{+}) mice, this study has demonstrated that COUP-TFII overexpression induced defects in the electron transport chain (ETC), downregulation of genes involved in mitochondrial fusion and quality control [such as mitofusin (Mfn)1 and Mfn2, optic atrophy 1 (Opa1), and PTEN-induced kinase 1 (Pink1)], accumulation of damaged mitochondria, and reduced mitochondrial reactive oxygen species (ROS) scavenging capacity, leading to oxidative stress and heart failure. Mitochondrial dysfunction induced by COUP-TFII overexpression may be mediated through the suppression of the PGC-1α-superoxide dismutase 2 (Sod2) pathway (69). Thus, alterations in the expression of key genes involved in both fatty acid and glucose metabolism by COUP-TFII may contribute to the development of metabolic inflexibility in heart failure (69). These findings are supported by gain- and loss-of-function models and transcriptomic data from two independent DCM cohorts (70,71). However, the limitations of these studies include the use of non-physiological overexpression levels, which may not accurately reflect COUP-TFII dysregulation in human disease, and a lack of longitudinal data to determine causality in patients (70,71). In addition, other COUP-TFII targets relevant to calcium handling, fibrosis or apoptosis were not explored and potential context-dependent roles of COUP-TFII in different tissues remain unresolved.

Increased COUP-TFII expression has been observed in diabetes-induced heart failure (DIHF) (72). Although metabolic disturbances, oxidative stress-induced cardiomyocyte death, inflammation and mitochondrial dysfunction are known to contribute to cardiac remodeling in DIHF, the pathophysiology and underlying mechanisms of DIHF remain unclear (73,74). A previous study demonstrated that the overexpression of COUP-TFII exacerbated DIHF by promoting ferroptosis and mitochondrial dysfunction, as evidenced by reduced mitochondrial damage and ferroptosis after COUP-TFII knockdown in palmitic acid (PA)-treated neonatal rat cardiomyocytes (NRCMs) (72). Ferroptosis, an iron-dependent, non-apoptotic form of cell death, is characterized by the accumulation of lipid ROS and mitochondrial overload (75). Although ferroptosis contributes to the development of CVDs and its suppression may alleviate diabetic myocardial ischemia-reperfusion injury (76-78) and cardiomyopathy (79), the role of mitochondria in ferroptosis remains controversial. Further studies are needed to validate the association between PA-induced ferroptosis and mitochondrial dysfunction in NRCMs. Taken together, the aforementioned studies suggest that COUP-TFII directly regulates mitochondrial metabolic regulator genes, including PPARα and PGC-1α (69), and its upregulation is associated with heart failure, possibly as a result of mitochondrial dysfunction (69,72). While the study effectively associates COUP-TFII with ferroptosis through lipid ROS and mitochondrial stress, its reliance on neonatal rat models limits its translational relevance (69). Additionally, the mechanistic role of mitochondria in ferroptosis is still open to debate (75-79), and the causal relationship between COUP-TFII upregulation and DIHF in human hearts is still unconfirmed. Further *in vivo* validation and exploration of the interaction of COUP-TFII with key mitochondrial regulators such as PPARα and PGC-1α are needed.

8. COUP-TFII in CRC

Tumor-suppressive role of COUP-TFII in CRC. Several studies have demonstrated that COUP-TFII acts as a tumor suppressor in CRC (80-83). A retrospective study using tissue microarray

assays have indicated that high COUP-TFII expression is associated with improved survival rates and reduced lymph node metastasis in patients with CRC; however, the initial study was limited by small sample sizes and short follow-up periods (80). These findings have subsequently been confirmed in larger cohorts with extended follow-up durations (81), supporting the hypothesis that COUP-TFII exerts tumor-suppressive effects in CRC.

Functional studies in human CRC cells have provided mechanistic insights. In SNU-C4 cells, COUP-TFII overexpression suppressed proliferation and invasion by upregulating the tumor suppressors p53 and PTEN, and by inhibiting Akt activity, respectively (82,83). Conversely, COUP-TFII knockdown in HT-29 CRC cells enhanced cell proliferation and invasive capabilities through the activation of the Akt pathway, leading to glycogen synthase kinase-3 β inactivation, β-catenin stabilization, and the upregulation of MMP7 and FOXC1 (83); however, the use of only two CRC cell lines (SNU-C4 and HT-29) limits the generalizability. These results underscore the function of COUP-TFII as a negative regulator of tumor aggressiveness in CRC. However, additional *in vivo* validation studies are necessary to confirm these findings and fully elucidate the mechanism by which COUP-TFII modulates CRC progression.

Tumor-promoting role of COUP-TFII in CRC. By contrast, a growing body of evidence indicates a tumor-promoting role of COUP-TFII under certain conditions. For example, patients with CRC with high COUP-TFII expression have an increased risk of metastasis and reduced overall survival, compared with patients with CRC with low COUP-TFII expression (84,85). Mechanistically, COUP-TFII directly binds to the promoter region of the *Snail1* gene, a master regulator of EMT, leading to the suppression of E-cadherin and increased immunosuppressive signaling (84,86-88). Additionally, miR-21, an oncomiR highly expressed in various types of cancer (89), increases COUP-TFII expression, which in turn promotes TGF-β-induced EMT by inhibiting Smad7 (90). COUP-TFII can also regulate the expression of miR-34a (91), a well-known tumor suppressor miRNA that inhibits tumor cell migration and promotes chemosensitivity (92-95). Notably, COUP-TFII-regulated miR-21 inhibits PTEN and elevates the levels of prostaglandin E_2, a pro-inflammatory and pro-tumorigenic mediator, by suppressing 15-hydroxyprostaglandin dehydrogenase, further contributing to tumor progression and inflammation (3,90). Although these findings are supported by cell and animal models, limitations remain, including reliance on overexpression systems, and unclear relevance across different types of cancer. A conflicting report of the anti-inflammatory roles of COUP-TFII in other tissues suggests context-dependent effects (96), emphasizing the need for further validation in human tumors and diverse inflammatory settings.

9. Dual functions of COUP-TFII as either a tumor suppressor or a tumor promoter

COUP-TFII exhibits context-dependent dual roles in CRC, functioning either as a tumor suppressor or as a tumor promoter, depending on the molecular and cellular environment (3,11). To illustrate these dichotomous roles, Table II summarizes the molecular and phenotypic outcomes associated with COUP-TFII expression in CRC.

The findings presented in Table II highlight the dualistic and context-dependent role of COUP-TFII in CRC, where its functional role is modulated by dynamic interactions with transcriptional regulators, non-coding RNAs and oncogenic signaling pathways.

As aforementioned, discrepancies in reported findings are possibly due to differences in study conditions, such as experimental models, patient cohorts and methodological approaches. Additionally, the majority of the aforementioned studies relied on *in vitro* and animal models, which may not fully recapitulate the pathophysiology of human CRC. Another limitation is the variability in COUP-TFII detection methods, such as differences in antibody specificity and RNA expression analysis techniques, which could contribute to inconsistent results. Future studies with standardized methodologies, large patient cohorts and human experimental data are needed to clarify the role of COUP-TFII in CRC.

10. Interrelation between CVDs and CRC

CVDs and CRC are among the leading causes of mortality worldwide (12). CRC is the third most common malignancy worldwide, accounting for 11% of all cancer diagnoses (97). Although CVDs and CRC are distinct disease entities, emerging evidence suggests that they may share common features (98,99). Using an ischemic cardiomyopathy C57BL/6J-ApcMin/J (APC^{min}) mouse model, researchers identified a potential causal association between heart failure and CRC by observing the association of presence of heart failure with enhanced tumor formation, possibly via cardiac excreted factors, such as SerpinA3, fibronectin and paraoxonase 1 (100). This study provided valuable insights into a potential causal association between heart failure and CRC, whereas its interpretation is still limited due to the use of a familial CRC model that does not fully recapitulate sporadic CRC, and the lack of mechanistic validation using targeted interventions, such as knockdown or neutralization of candidate cardiac excreted factors. In a clinical study, patients aged >65 years with stage I-III CRC were revealed to have a higher risk of developing new-onset CVDs compared to a matched cohort of Medicare patients without cancer (13), suggesting the possibility of shared vulnerability or cancer treatment-related cardiovascular complications. However, the observational nature of the study lacks causal inference, while the residual confounding effect of common risk factors (such as age, diabetes and smoking) cannot be excluded. Two meta-analyses have demonstrated that use of angiotensin converting enzyme inhibitors/angiotensin receptor blockers and renin-angiotensin system (RAS) inhibitors was associated with a decreased risk of CRC, respectively (101,102). These findings indicate that hypertension might be a causal factor for CRC. Although these findings are promising, the analyses are limited by heterogeneity in study design, population characteristics and potential indication bias, since patients receiving RAS inhibitors may be healthier or more closely monitored than non-users. Furthermore, emerging evidence indicates that chronic inflammation and oxidative stress, the underlying pathophysiological

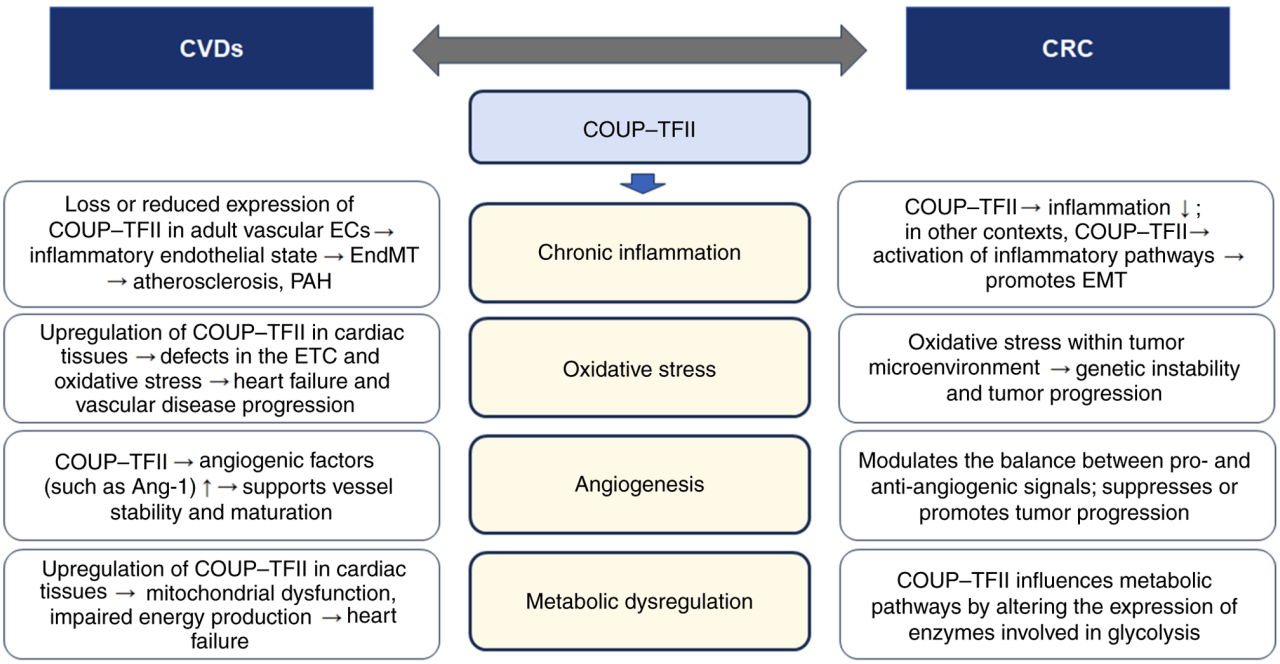

Figure 2. Schematic illustration of the molecular mechanisms by which COUP-TFII functions as a common regulator in both CVDs and CRC. Although CVDs and CRC are distinct disease entities, they share common pathophysiological features. In particular, chronic inflammation, oxidative stress, angiogenesis and metabolic dysregulation by COUP-TFII may serve roles in the pathogenesis of both conditions. COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; EC, endothelial cell; EndMT, endothelial-to-mesenchymal transition; ETC, electron transport chain; Ang-1, angiopoietin-1; EMT, epithelial-to-mesenchymal transition; CVD, cardiovascular disease; CRC, colorectal cancer; PAH, pulmonary arterial hypertension.

mechanisms for both diseases, may contribute to the association between CVDs and CRC (16,103,104). Additional mechanisms that have been suggested include the cardiotoxicity of certain anticancer agents, shared molecular pathways between the two diseases and innate immunity (103,105-107). However, while these mechanisms are supported by both experimental and clinical evidence (16,103-107), the evidence remains largely correlative and prospective studies with mechanistic endpoints are needed to clarify their roles and causality.

COUP-TFII as a common molecular regulator of CVDs and CRC. Despite the controversial roles of COUP-TFII in CRC, growing evidence (3,5,20,46,69,82,89,90) has associated chronic inflammation, oxidative stress, angiogenesis and metabolic dysregulation with both CVDs and CRC, with COUP-TFII serving as a common molecular regulator. Fig. 2 illustrates the molecular mechanisms by which COUP-TFII functions as a common regulator in both CVDs and CRC.

Based on published reports (46,108) and the aforementioned descriptions, chronic inflammation is involved in both CVDs and CRC (Fig. 2). As previously mentioned (46), COUP-TFII is key to maintain endothelial homeostasis. In adult vascular ECs, it represses the expression of pro-inflammatory cytokines (such as CXCL10, CXCL11 and CCL5), while supporting the expression of anti-thrombotic genes, such as TFPI2. Loss or reduced expression of COUP-TFII can lead to an inflammatory endothelial state, triggering EndMT, which contributes to atherosclerosis and other vascular pathologies (46). In the tumor microenvironment of CRC, chronic inflammation is a known driver of cancer progression (108). COUP-TFII appears to serve a dual role, depending on the cellular context. In some CRC cells, overexpression of COUP-TFII promotes tumor-suppressive signaling by upregulating p53 and PTEN while inhibiting Akt (82), which can help reduce inflammation. However, in other contexts, increased COUP-TFII expression, especially when upregulated by miRNAs such as miR-21, can promote EMT through activation of inflammatory pathways, thereby enhancing tumor invasiveness (3,89,90).

Oxidative stress serves an important role in both CVDs and CRC (Fig. 2). In the heart and vasculature, COUP-TFII is involved in the regulation of mitochondrial function. Upregulation in cardiac tissues has been associated with defects in the ETC, and a reduction in the expression of mitochondrial quality control genes (such as Mfn1, Mfn2, Opa1 and Pink1). This leads to the accumulation of damaged mitochondria and impaired ROS scavenging, culminating in elevated oxidative stress, a key factor in heart failure and vascular disease progression (69). While the study by Wu *et al* (69) is pivotal in terms of mechanistic exploration using transgenic mouse models of DCM, some limitations should be addressed. First, the cardiac-specific effects of COUP-TFII may not be directly generalizable to other tissues, including the colon. Second, although the mitochondrial dysfunction and oxidative stress were associated with COUP-TFII levels, direct causality and dose-response relationships were not fully established, particularly with respect to downstream disease phenotypes. In CRC, oxidative stress within the tumor microenvironment can promote genetic instability and tumor progression (109). However, lack of direct evidence associating COUP-TFII with ROS regulation in CRC leaves the proposed relationship speculative. To the best of our knowledge, the majority of the existing data, including the aforementioned study by Yang *et al* (109), focus on general mechanisms of

ROS in cancer, and do not specifically implicate COUP-TFII. Therefore, while it is plausible that regulation of metabolic and mitochondrial pathways by COUP-TFII could indirectly influence ROS levels in CRC, this hypothesis remains unvalidated in CRC-specific *in vivo* models. Further research using CRC-specific conditional knockout or overexpression models is essential to clarify whether COUP-TFII modulates oxidative stress in the colon epithelium or tumor microenvironment and whether this mechanism contributes causally to colorectal tumorigenesis.

COUP-TFII regulates angiogenesis, affecting the progression of both CVDs and CRC (Fig. 2). COUP-TFII positively regulates angiogenic factors such as Ang-1 by binding to its promoter (often in cooperation with Sp1), which supports vessel stability and maturation (46). This function is key for normal vascular repair and to prevent pathological calcification (46). In CRC, COUP-TFII modulates the balance between pro- and anti-angiogenic signals. By influencing the expression of factors that control angiogenesis, COUP-TFII can impact tumor vascularization (17,20). Depending on the cellular context, this modulation may either suppress or promote tumor progression.

COUP-TFII also regulates energy metabolism, influencing both cardiac function and cancer cell proliferation (Fig. 2). COUP-TFII regulates various genes such as *GLUT4*, *PPARα*, fatty acid binding protein 3 and carnitine palmitoyltransferase 1 involved in both fatty acid and glucose metabolism (3,5). Upregulation of COUP-TFII in cardiac tissues has been associated with the downregulation of key metabolic regulators, such as PGC-1α and Sod2, leading to mitochondrial dysfunction, impaired energy production and ultimately heart failure (69). In CRC, COUP-TFII can influence metabolic pathways through its effects on the Akt signaling cascade and by altering the expression of enzymes involved in glycolysis (5). This metabolic dysregulation provides the energetic and biosynthetic requirements for rapid tumor cell proliferation, thereby contributing to cancer progression.

11. Therapeutic implications of COUP-TFII in CVDs

Vascular interventions using grafts are often required to treat severe CVDs (110). However, traditional grafts face challenges, such as susceptibility to infection, thrombogenicity and poor long-term patency rates (111-113). Recent advances in vascular biology underscore the key role of COUP-TFII in regulating endothelial identity and angiogenesis, protecting against atherosclerosis, and mitigating vascular calcification (114). Xing *et al* (114) demonstrated that COUP-TFII overexpression following the localized delivery of COUP-TFII pDNA nanocarriers (COUP-TFII@HPEI) in a rat abdominal artery replacement model regulated stem/progenitor cell differentiation towards endothelialization, and inhibited the calcification of decellularized allografts. This presents an effective strategy to enhance the applicability of decellularized allografts for vascular interventions (114). However, the limitations of this study include the use of a short-term preclinical model, and the lack of long-term patency and immune response data. Furthermore, the effect of COUP-TFII overexpression on systemic metabolism and off-target tissues was not assessed. Wu *et al* (69) revealed that reducing the COUP-TFII expression in *calcineurin* (CnTg/Cre/F^+) transgenic mouse models that develop DCM resulted in an increase in overall survival. Thus, inhibiting COUP-TFII to restore the metabolic balance in the heart exhibits potential as a therapeutic approach for heart failure, highlighting the potential of COUP-TFII in CVD treatments. Nevertheless, there are also limitations to this study, including its reliance on a genetically engineered model with a strong calcineurin-dependent phenotype, which may not generalize to other forms of heart failure, and the use of global or tissue-specific knockouts without temporal control of gene expression. Overall, these findings emphasize the context-dependent effects of COUP-TFII: While its overexpression may support vascular regeneration and graft compatibility by enhancing endothelial reprogramming, it may conversely exacerbate cardiac dysfunction when dysregulated in the myocardium. This dichotomy emphasizes the importance of spatiotemporal control in potential COUP-TFII-targeted therapies. Additional studies are warranted to resolve these paradoxes, validate the efficacy and safety in large-animal models or human tissues, and establish optimal delivery strategies that minimize off-target effects, while leveraging the therapeutic potential of the gene of interest.

12. Therapeutic implications of COUP-TFII in CRC

The role of COUP-TFII in CRC remains controversial. Therefore, targeting COUP-TFII in CRC requires further exploration. In tumor-promoting contexts of COUP-TFII, small molecules or RNA-based therapies targeting COUP-TFII are anticipated to show promise, according to previous preclinical models such as prostate cancer xenograft models and patient-derived xenograft mice (115). Qin *et al* (20) revealed that the inhibition of COUP-TFII via conditional ablation in a mouse xenograft model resulted in impaired neoangiogenesis and suppressed tumor growth. Although this study provided a mechanistic explanation for the angiogenic role of COUP-TFII, its dependence on immune-deficient mouse models may limit translational relevance, particularly in the context of tumor-immune interactions (20). Additionally, Wang *et al* (116) revealed that COUP-TFII knockdown attenuated tumorigenesis and tumor progression in an immunocompetent mouse glioblastoma model, indicating that COUP-TFII may have a broader oncogenic function in various types of cancer. However, the results are still based on a tumor type-specific model and lack CRC-specific validation. Wang *et al* (115) identified a small molecular inhibitor of COUP-TFII using a luminescence-based cell-based high-throughput screening assay, and also revealed that this inhibitor reduced prostate cancer cell proliferation, colony formation, cell invasion and angiogenesis in xenograft mouse models and patient-derived xenograft models. Although promising, the selectivity, bioavailability and toxicity profiles of the compound were not fully addressed, and its effects in CRC models remain unknown. These findings support the rationale for developing COUP-TFII-targeted agents, particularly in tumors with COUP-TFII upregulation. Similarly, targeting COUP-TFII to inhibit angiogenesis and metabolic reprogramming may suppress tumor progression. To the best of our knowledge, no clinical trials specifically targeting

COUP-TFII have been carried out; however, related nuclear receptor-targeting compounds, such as retinoic acid receptor agonists (117) and HDAC inhibitors (40), are being investigated in cancer therapy and may provide insights into potential COUP-TFII-targeted approaches.

On the other hand, there is evidence that COUP-TFII expression is associated with certain positive prognostic indicators in CRC, suggesting its potential role as a tumor suppressor (80,81). Therefore, extensive prospective studies are necessary to reconcile these conflicting observations. Kruse *et al* (18) reported the potential of retinoic acids as COUP-TFII promoters using multiple cell line experiments; however, the required concentration was greater than the physiological levels of retinoic acids, raising concerns regarding clinical feasibility. In tumor-suppressive contexts, developing novel drugs that can enhance COUP-TFII expression may provide novel therapeutic avenues for CRC treatment.

In terms of synergy with existing treatments (for example, doxorubicin or 5-fluorouracil), COUP-TFII modulation has the potential to enhance the efficacy of standard chemotherapy and targeted therapies (118). For example, several studies have suggested that COUP-TFII modulates Wnt/β-catenin and TGF-β signaling pathways, including EMT, both of which are responsible for resistance to conventional CRC treatments (84,89,118). In particular, the reduction of COUP-TFII after doxorubicin treatment may contribute to EMT-induced doxorubicin resistance in CRC cells (118), supporting the possibility that COUP-TFII upregulation can lead to chemosensitivity to doxorubicin in CRC. Taken together, combining COUP-TFII modulators with conventional Wnt pathway inhibitors or immune checkpoint inhibitors may improve treatment responses in resistant tumors by hindering resistance mechanisms. Furthermore, given the role of COUP-TFII in angiogenesis and metastasis, its inhibition could complement anti-angiogenic therapies, such as VEGF inhibitors, by preventing tumor vascularization and dissemination (17,20). However, challenges remain in translating these findings into clinical applications.

13. Future research directions

There are several challenges in research that should be overcome in developing COUP-TFII-related therapies for CVDs and CRC. One major concern is the lack of well-characterized small-molecule inhibitors or modulators that can specifically target COUP-TFII without affecting other nuclear receptors. Additionally, the dual role of COUP-TFII as both an oncogene and tumor suppressor in different contexts complicates therapeutic strategies for CRC, underscoring the need to investigate potential biomarkers to identify patients who would benefit from COUP-TFII-targeted interventions. Furthermore, the development of selective COUP-TFII modulators, optimization of drug delivery systems and comprehensive preclinical validation are necessary steps before clinical trials can be pursued.

Therefore, future research should focus on elucidating the precise regulatory mechanisms of COUP-TFII, identifying specific patient subgroups that would benefit from COUP-TFII-targeted therapies, carrying out human clinical trials based on previous preclinical experimental data, exploring combination strategies to enhance treatment efficacy while minimizing toxicity, and developing personalized treatments based on these findings. Structural studies on the LBD of COUP-TFII may help identify novel therapeutic ligands or modulators. Investigating the interactions of COUP-TFII with key signaling molecules, such as VEGF and PGC-1α, could further help identify potential therapeutic targets. Additionally, understanding the interaction between COUP-TFII-related CVDs and CRC will be key to develop integrated therapeutic approaches. For example, longitudinal studies examining the bidirectional risk between CVDs and CRC could provide valuable insights into their shared pathophysiological mechanisms. Biomarker-based approaches to monitor COUP-TFII activity could also guide tailored treatment strategies for patients with coexisting CVDs and CRC.

14. Conclusion

COUP-TFII is a versatile transcription factor with key roles in both CVDs and CRC. Its structural features and regulatory mechanisms enable it to affect diverse cellular processes, from angiogenesis to metabolism. Future studies should aim to uncover the tissue-specific roles of COUP-TFII in various pathological contexts, develop selective modulators of COUP-TFII for clinical applications, and explore the interplay between COUP-TFII-mediated pathways in CVDs and CRC. Comprehensive understanding of the dual roles of COUP-TFII in CRC and its interrelation with CVDs, will provide opportunities for innovative therapeutic strategies that target this nuclear receptor.

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Availability of data and materials

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Authors' contributions

THP and JIP were responsible for conception, organization, searching the literature, writing of the first draft and reviewing the manuscript. SHH, JGC, SJP and JYH searched the literature and reviewed the manuscript. SHH and JIP designed the figures. Data authentication is not applicable. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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