Multiple roles of COUP-TFII in cancer initiation and progression

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Abstract

Chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) is an orphan nuclear receptor that acts as a transcriptional activator or repressor in a cell type-dependent manner. Best characterized for its role in the regulation of angiogenesis during mouse development, COUP-TFII also plays important roles in glucose metabolism and cancer. Expression of COUP-TFII is altered in various endocrine conditions. Cell type-specific functions and the regulation of COUP-TFII expression result in its varying physiological and pathological actions in diverse systems. Evidence will be reviewed for oncogenic and tumor-suppressive functions of COUP-TFII, with roles in angiogenesis, metastasis, steroidogenesis, and endocrine sensitivity of breast cancer described. The applicability of current data to our understanding of the role of COUP-TFII in cancer will be discussed.

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Introduction

Steroid hormones and nuclear receptor (NR) ligands play critical roles in cancer initiation and progression, and their antagonists have proven efficacy in the treatment and prevention of cancers. This is most notable in breast and prostate cancers and the use of all-trans retinoic acid (RA) for acute promyelocytic leukemia (Risbridger et al. 2010, Siddikuzzaman et al. 2011). Steroid/NRs act as ligand-activated transcription factors to either positively or negatively regulate gene expression (Stanisic et al. 2010, Ahmad & Kumar 2011). Activation of NRs occurs through binding a variety of ligands including hormones and vitamins/retinoids. NRs have physiological roles to modulate gene expression during development and growth. As alteration of basal gene expression leads to many pathogenic outcomes, including cancer, maintenance of normal gene expression by NRs is vital. One such critical NR is chicken ovalbumin upstream promoter transcription factor II (COUP-TFII). From the time of the identification of the COUP-TF family in 1986 (Sagami et al. 1986), the many functions of COUP-TFs have continued to be explored. The role of COUP-TFII in cancer is widely debated with evidence linking COUP-TFII to both tumor-suppressive and oncogenic functions. This review will explore both the regulation and function of COUP-TFII and its connections to cancer.

COUP-TFI and COUP-TFII

The COUP-TF family consists of two highly homologous subtypes, COUP-TFI and COUP-TFII, located on human chromosomes 5 and 15 respectively (Fig. 1). COUP-TFs have been previously reviewed (Tsai & Tsai 1997, Lin et al. 2011), but not in the specific context of separating COUP-TFI and COUP-TFII in cancer. COUP-TFs are ancient NRs and are located close to retinoid X receptors (RXRs) in the evolutionary tree (Thornton 2001, Thornton et al. 2003). As evolutionarily conserved transcription factors, COUP-TFs have major roles in development. The importance of COUP-TFII expression is evidenced by studies in knockout mice (Pereira et al. 1999). Homozygous mutation of COUP-TFII (Nr2f2) leads to embryonic lethality due to impaired angiogenesis and heart defects, resulting in hemorrhage and edema. These effects may in part be explained by the reduction in angiopoietin-1 (Ang-1) expression in COUP-TFII-null mice (Pereira et al. 1999). Other important embryonic roles for COUP-TFII include regulation of limb growth and muscle development (Lee et al. 2004). COUP-TFII-null mice display a reduction in expression of Lhx1, a protein required for proper muscle precursor cell migration, and in myogenin, which is necessary for muscle cell differentiation (Lee et al. 2004, Vasyutina & Birchmeier 2006).
Based on the high-sequence identity in their DNA binding domains (Fig. 1), we anticipate that COUP-TFI and COUP-TFII regulate the same genes. However, this has not been empirically tested and it is worth noting that the N-terminus is divergent (Fig. 1) and immunoprecipitation studies indicate differences in proteins interacting with COUP-TFI (Zhang et al. 2009) and COUP-TFII (Litchfield et al. 2012), although, again, this has not been systematically studied in cells in which both are expressed. COUP-TFI and COUP-TFII may have divergent functions in certain contexts as well. Differences in COUP-TFI and COUP-TFII function in breast cancer endocrine sensitivity, for example, have also been identified (Riggs et al. 2006). This review will focus specifically on COUP-TFII.

**COUP-TFII regulation of gene expression**

**Mechanisms of regulation**

COUP-TFII can activate or repress gene expression in both a tissue-specific and gene-specific manner through mechanisms involving direct binding to DNA response elements or binding to other transcription factors. Through binding to 5' AGGTCA-3' direct repeats (DRs) with variable spacing (Kliewer et al. 1992), COUP-TFII modulates the expression of target genes. Specific genes upon which COUP-TFII activates transcription include RA receptor β2 (RARβ2, RARB2; Lin et al. 2000, Litchfield et al. 2012), phosphoenolpyruvate carboxykinase (PEPCK, PCK1; De Martino et al. 2004a,b), NGFI-A (Egr1; Pipaon et al. 1999, Kruse et al. 2008), and cholesterol 7α-hydroxylase (CYP7A1; Stroup & Chiang 2000). COUP-TFII action may be potentiated by interaction with coactivators such as steroid receptor coactivator family members SRC-1/NCOA1, SRC-2/NCOA2, and SRC-3/NCOA3 (Pipaon et al. 1999, Kruse et al. 2008), as well as PGC1α (Kruse et al. 2008), p300/CBP (Pipaon et al. 1999), orphan receptor coactivator (ORCA; Marcus et al. 1996), and nucleolin (Litchfield et al. 2012). DNA binding of COUP-TFII can promote the binding of a second transcription factor, further activating gene transcription. This occurs for both the PCK1 and Cyp7a1 genes, where COUP-TFII binding to the promoter recruits binding of glucocorticoid receptor (GR) to enhance gene expression (De Martino et al. 2004a,b). COUP-TFII can also bind to Sp1 sites to cooperatively activate gene expression, as was reported for regulation of Otx2 expression during morphogenesis in the mouse eye (Tang et al. 2010).

Alternatively, binding of COUP-TFII to DRs may result in repression of gene expression. In the mechanism of ‘active repression,’ COUP-TFII binding results in recruitment of corepressors, i.e. nuclear corepressor (NCoR; Bailey et al. 1997) and silencing mediator of retinoid and thyroid receptors (SMRTs; Shibata et al. 1997, Okamura et al. 2009), resulting in repressed chromatin structure and a corresponding blockade of target gene transcriptional activation. COUP-TFII interaction with SMRT represses PPARγ1 (Pparg1) and PPARγ2 (Pparg2) expression to suppress adipogenesis (Okamura et al. 2009). Repression of the human oxytocin promoter by COUP-TFII binding has also been reported (Chu & Zingg 1997). COUP-TFII represses PAX6 expression in the retina via binding to a DRI site (TGTCACAGTCCA; Tang et al. 2010).

Through an alternative mechanism of transrepression, COUP-TFII can interact with other NRs and transcription factors to inhibit their normal transcriptional activity. Examples of this include inhibition of ER- and GR-induced gene expression in a gene-specific manner (Klinge et al. 1997, De Martino et al. 2004b). COUP-TFII can also repress AP-1 signaling through interaction with c-Jun (Lin et al. 2002). Interaction of COUP-TFII with Runx2 inhibits osteoblast differentiation via blocking Runx2 binding to the osteocalcin promoter (Lee et al. 2012). Other mechanisms of repression involve the modulation of ER, RXR, PPAR, and VDR activity by competing for DNA response element binding or heterodimerization with the class II heterodimeric partner RXR (Cooney et al. 1993).

*COUP-TFI and cancer*
<table>
<thead>
<tr>
<th>Gene (protein)</th>
<th>Name</th>
<th>Location</th>
<th>Family</th>
<th>Regulation by COUP-TFII</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALDH2</td>
<td>Aldehyde dehydrogenase 2 family (mitochondrial)</td>
<td>Cytoplasm</td>
<td>Enzyme</td>
<td>Decrease</td>
<td>You et al. (2002)</td>
</tr>
<tr>
<td>ANGPT1</td>
<td>Angiopoietin 1</td>
<td>Extracellular space</td>
<td>Growth factor</td>
<td>Increase</td>
<td>Pereira et al. (1999)</td>
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<tr>
<td>APOA1</td>
<td>Apolipoprotein A-I</td>
<td>Extracellular space</td>
<td>Transporter</td>
<td>Decrease</td>
<td>Widom et al. (1992), Jiang et al. (1995) and Power &amp; Cereghini (1996)</td>
</tr>
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<td>Ochoa et al. (1993) and Sauvaget et al. (2002)</td>
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<tr>
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<td>Transporter</td>
<td>Decrease</td>
<td>Mietus-Snyder et al. (1992), Power &amp; Cereghini (1996), Ktistaki &amp; Talianidis (1997) and Laurantiadou et al. (1999)</td>
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<td>GATA6</td>
<td>GATA binding protein 6</td>
<td>Nucleus</td>
<td>Transcription regulator</td>
<td>Decrease suggested</td>
<td>Kymizi et al. (2006)</td>
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<td>Cytoplasm</td>
<td>Transporter</td>
<td>Decrease</td>
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<td>Transcription regulator</td>
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<td>HNF1 homeobox B</td>
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<td>Transcription regulator</td>
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<tr>
<td>HNF4A</td>
<td>Hepatocyte nuclear factor 4, alpha</td>
<td>Nucleus</td>
<td>Transcription regulator</td>
<td>Increase</td>
<td>Perlou et al. (2008)</td>
</tr>
<tr>
<td>KDR</td>
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<td>Kinase</td>
<td>Decrease</td>
<td>Kang et al. (2010)</td>
</tr>
<tr>
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<td>Enzyme</td>
<td>Decrease</td>
<td>Rufibach et al. (2006)</td>
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<tr>
<td>LPL</td>
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<td>Cytoplasm</td>
<td>Enzyme</td>
<td>Increase</td>
<td>Robinson et al. (1999)</td>
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<td>Peptidase</td>
<td>Decrease</td>
<td>Lee et al. (1995)</td>
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<td>Other</td>
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<td>Yu et al. (1998)</td>
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<td>NR5A2</td>
<td>LRH1, nuclear receptor subfamily 5, group A, member 2</td>
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<td>Plasma membrane</td>
<td>Transmembrane receptor</td>
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<td>Kang et al. (2010)</td>
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<td>Cytoplasm</td>
<td>Kinase</td>
<td>Decrease</td>
<td>Eubank et al. (2001)</td>
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<td>POUF1</td>
<td>Oct 4; POU class 5 homeobox 1</td>
<td>Nucleus</td>
<td>Transcription regulator</td>
<td>Decrease</td>
<td>Ben-Shushan et al. (1995) and Rosa &amp; Brivanlou (2011)</td>
</tr>
</tbody>
</table>
Ingenuity Pathway Analysis

As summarized here, COUP-TFI I regulates the expression of diverse gene targets. Table 1 contains a list of known COUP-TFI I targets as identified using Ingenuity Pathway Analysis (IPA; Ingenuity Systems, www.ingenuity.com). These targets are also displayed in Fig. 2. COUP-TFI I has varying effects on expression of other NRs and transcription factors. COUP-TFI I increased the expression of HNF1α (Ktistaki & Talianidis 1997), HNF1β (Power & Cereghini 1996), HNF4α (Perilhou et al. 2008b), and RARβ (Wu et al. 1997, Lin et al. 2000, Litchfield et al. 2012), while it decreased the expression of Oct4 (Ben-Shushan et al. 1995, Rosa & Brivanlou 2011), Dax1 (Yu et al. 1998), and PPARα (Pineda Torra et al. 2002). As previously described, COUP-TFI I has well-known functions in repressing the transcriptional activity of other NRs and transcription factors. Although COUP-TFI I increases HNF4 expression, other reports highlight the repression of HNF4 function by COUP-TFI I. Specifically, COUP-TFI I decreases transcriptional activation of ALDH2 (You et al. 2002) and retinol binding protein 2 (RBP2; Nakshatri & Chambon 1994) by HNF4. The HNF4 activation of hepatic lipase is suppressed by COUP-TFI I (Rufibach et al. 2006), while lipoprotein lipase expression is induced by COUP-TFI I synergistically with PPARγ (Robinson et al. 1999), part of the many of reported functions of COUP-TFI I in the cholesterol-processing pathway. A similar response occurs for apolipoproteins A-I, A-IV, and C-III, where COUP-TFI I represses the RXRα-mediated expression of APOA-I (Widom et al. 1992, Jiang et al. 1995, Power & Cereghini 1996) and HNF4-mediated expression of APOA-IV (Ochoa et al. 1993, Sauvaget et al. 2002) and APOC-III (Mietus-Snyder et al. 1992, Power & Cereghini 1996, Ktistaki & Talianidis 1997, Lavrentiadou et al. 1999). HNF4 and COUP-TFI I binding to the sex hormone binding globulin (SHBG) promoter was reported in murine Sertoli cells (Selva et al. 2005). SHBG expression is increased by HNF4 and suppressed by COUP-TFI in HepG2 hepatoblastoma cells (Janne & Hammond 1998). Decreased SHBG expression is indicative of metabolic syndrome and may result in increased plasma androgen and estrogen levels, although the precise connection of COUP-TFI I to these phenotypes has not been investigated (Hammond 2011).

Although COUP-TFI I is classically known for its role in transrepression, COUP-TFI I may also enhance the effect of a second NR. Induction of cytochrome P450 family members cholesterol 7α-hydroxylase CYP7A1 (Stroup & Chiang 2000) and aldosterone synthase CYP11B2 (Shibata et al. 2004, Kurihara et al. 2005) by COUP-TFI I was reported, with COUP-TFI I and HNF4 acting to synergistically activate CYP7A1 (Stroup...
CYP7A1 catalyzes the first step in the conversion of cholesterol to bile acid (Stroup & Chiang 2000), while CYP11B2 catalyzes the final steps of aldosterone synthesis (Kurihara et al. 2005), implying that COUP-TFII transcriptional activation would increase the production of bile acid and aldosterone.

As shown in Table 1 and Fig. 2, COUP-TFII opposes PPARγ/RXR activation of PEPCK transcription in preadipocytes/fibroblasts, a result that was proposed to suppress adipogenesis (Eubank et al. 2001). COUP-TF also inhibited 9-cis RA/RXR-induced activation of the lactotransferrin promoter in transiently transfected ZR-75-1 and Hs578T breast cancer cells apparently by competing for DNA binding to a composite RARE/ERE in the gene promoter (Lee et al. 1995). Concurrent binding of COUP-TFII and NF-Y to the hemoglobin epsilon promoter leads to a repression of gene expression (Liberati et al. 2001). In addition to the targets identified by IPA, COUP-TF was reported to play a dual regulatory role in the transcriptional regulation of the mitochondrial HMG-CoA synthase gene: alone COUP-TFII stimulated reporter gene activity from the HMG-CoA synthase promoter in transiently transfected HepG2 human hepatoma and rat Leydig tumor R2C cells, but it inhibited PPARγ-stimulated transcriptional activity by competing for the same DNA binding site (Rodriguez et al. 1997).

Some of the IPA-identified COUP-TFII target gene relationships and mechanisms remain to be fully elucidated. In a study of the transcriptional regulation of murine hepatic development, COUP-TFII occupancy of Gata6, Fxr (Nr1h4), Pxr (Nr1i2), and Lrh1 (Nr5a2) promoters, as determined by chromatin immunoprecipitation (ChIP) assay, was reported during the postnatal period (Kyrmizi et al. 2006). While an inhibitory relationship was suggested for the effect of COUP-TFII on GATA6, the effect on FXR, PXR, and LRH1 expression is not yet known (Kyrmizi et al. 2006).

Several other target genes have been identified that highlight the critical function of COUP-TFII in the vascular system. These include an increase in Ang-1 (Pereira et al. 1999) and natriuretic peptide A (Higgins et al. 2001) by COUP-TFII and a decrease in VEGFR2 and neuropilin 1 (Kang et al. 2010). COUP-TFII enhances expression of the NHE1 solute exchanger (Fernandez-Rachubinski & Fliegel 2001, Li et al. 2002).
In summary, as indicated by the IPA (Fig. 2) and consistent with previous reports, COUP-TFII plays a role in many downstream pathways and may either activate or suppress gene expression.

Role in the RA pathway

COUP-TFs are classified as orphan members of the NR superfamily because their endogenous ligand(s) is not known. However, Kruse et al. (2008) demonstrated in silico binding of all-trans (atRA) and 9-cis (9cRA) RA to the crystal structure of the COUP-TFII ligand-binding domain (LBD). RA released the COUP-TFII LBD from the auto-repressed conformation. While the investigators did not directly test binding of all-trans or 9-cis RA to COUP-TFII, they demonstrated that treatment with atRA or 9cRA increased COUP-TFII interaction with the coactivator SRC-3, with an EC50 of 10–30 μM. In agreement with this data, addition of 20 μM atRA or 9cRA led to COUP-TFII’s activation of a NGFI-A-luciferase reporter (Kruse et al. 2008). Although these concentrations of atRA and 9cRA are greater than the physiological concentration of these retinoids, this finding provides novel insight into the ligand binding ability of COUP-TFII. Indeed, the function of this activation can be seen in the regulation of RARβ2 by COUP-TFII, as COUP-TFII activation of RARβ2 expression is increased with the addition of all-trans RA (Lin et al. 2000, Litchfield et al. 2012). Treatment of MCF-7 breast cancer cells with atRA also increased COUP-TFII-binding to the RARB2 promoter in a ChIP assay (Litchfield et al. 2012). RA induces the expression of COUP-TFII in certain breast cancer cell lines (e.g. T47D and ZR-75) but not others (e.g. MCF-7 and MDA-MB-231) (Fig. 3) (Nakshatri et al. 2000, Litchfield et al. 2012). This indicates a potential feed-forward loop, as treatment with RA may increase both the expression and activation of COUP-TFII, with downstream effects on RAR.

Regulation of COUP-TFII expression

Tissue-specific regulation in humans

COUP-TFII has a widespread tissue distribution, with detectable expression in every human tissue type examined (Suzuki et al. 2000a). The regulation of COUP-TFII expression is tissue and cell-type specific and can be modulated both transcriptionally and posttranscriptionally (Fig. 3). Hyperinsulinemia is a risk for breast cancer (Gunter et al. 2009, Ferguson et al. 2012). COUP-TFII expression was repressed by insulin and glucose in the liver and pancreas of C57BL6/J mice and in mouse primary hepatic and pancreatic cell culture (Perilhou et al. 2008a). By contrast, we found that insulin treatment had no effect on COUP-TFII expression in MCF-7 and T47D breast cancer cells (Fig. 4). The lack of alteration in COUP-TFII expression with insulin in breast cancer cells highlights the importance of cell-specific regulation of COUP-TFII expression. There are currently no reports on the effect of insulin on COUP-TFII expression in other cancers.

MicroRNA regulation

MicroRNA (miRNA) expression is altered in a variety of conditions and disease states, including cancer, and results in important posttranscriptional regulation of crucial proteins (Lovat et al. 2011). While 115 miRNAs are predicted to target NR2F2 (http://cometa.tigem.it/site/index.php), only one miRNA has been verified. miRNA-302 directly represses COUP-TFII expression in human embryonic stem cells (Rosa & Brivanlou 2011). Regulation of COUP-TFII expression by miRNA has not yet been reported in cancer cells.

DNA methylation

Methylation at CpG islands can result in suppression of gene transcription and is known to be a hallmark of cancer progression. DNA methylation may also occur at intragenic and intergenic sites, as well as at the promoter (Deaton & Bird 2011, Shenker & Flanagan 2012). Specifically, COUP-TFII has been found to be methylated in many cancers, including mantle cell lymphoma, acute myeloid leukemia, salivary gland adenoid cystic carcinoma, pancreatic adenocarcinoma, colon cancer, breast cancer ductal carcinoma in situ, as well as a tamoxifen-resistant breast cancer cell line (Fan et al. 2006, Irizarry et al. 2009, Tommasi et al. 2009, 2012).
expression due to epigenetic modification across cancer types remains to be seen. Contrary to these reports, high levels of COUP-TFII mRNA expression were found in all cell lines in the NCI60 panel of human cancer cell lines (Holbeck et al. 2010).

Regulation by other transcription factors

COUP-TFII and Ets-1 have overlapping expression patterns in mesenchymal cells of the mouse gut, spleen, lungs, and other tissues (Petit et al. 2004). Members of the ETS family (Ets-1, Ets-2, ETV, PEA3, Spi-1, and ERM) increased murine COUP-TFII-promoter activity in HeLa cells. Steroid receptor coactivators SRC-1/NCOA1, TIF2/SRC-2/NCOA2, and RAC3/SRC-3/NCOA3 enhanced the activation of the COUP-TFII promoter (Petit et al. 2004). In agreement with these data, SRC-3 and RARα increased COUP-TFII-promoter activity in HepG2 human hepatocellular carcinoma cells with atRA treatment. Reciprocally, siRNA knockdown of SRC-3 repressed COUP-TFII expression (Ma et al. 2011). We observed that the protein expression (by immunohistochemical staining) of AIB1/SRC-3/NCOA3, PEA3, and SRC-1/NCOA1 was correlated with COUP-TFII in breast cancer patient samples (Litchfield et al. 2012).

Regulation by altered kinase activity and other signaling pathways

Several factors were reported to alter COUP-TFII expression in pathogenic states. More et al. reported that expression of COUP-TFII, but not COUP-TFI, is stimulated by activation of the MAPK pathway. Breast cancer cell lines with increased MAPK activity, i.e. SKBR3, had a concomitant increase in COUP-TFII expression (More et al. 2003). In contrast to the idea that MAPK activation increases COUP-TFII expression, MAPK has also been shown to phosphorylate and inactivate protein phosphatase 2A (PP2A), leading to a suppression of COUP-TFII expression in human peripheral blood CD34+ cells (Aerbajinai et al. 2009). PP2A is inhibited by the FOXO transcription factors, including FOXO1 (Ni et al. 2007). COUP-TFII expression is induced by FOXO1 in pancreatic β cells and hepatocytes (Perilhou et al. 2008a), highlighting the highly cell type-specific nature of these pathways. MAPK activity may lead to increased COUP-TFII expression in certain conditions, while it may alternatively repress COUP-TFII in others. Taken together, these data suggest a possible feedback loop in certain cell types (Fig. 3).
In addition to MAPK activation, Notch signaling is also dysregulated in many types of cancer. Increased Notch signaling has been implicated in carcinogenesis and metastasis and is also involved in regulation of endothelial cell proliferation and angiogenesis (Garcia & Kandel 2012, Gu et al. 2012). In breast cancer, Notch and its ligand Jagged1 upregulate the expression of Slug, a transcriptional repressor of E-cadherin important in metastatic progression (Leong et al. 2007). Notch signaling has also been implicated in the amplification of HER2 and survival of tumor initiating cells of both arterial and venous origin and in mouse studies (You et al. 2005, Kang et al. 2010, Srinivasan et al. 2010). Notch can suppress COUP-TFII and Prospero-related homeobox domain 1 (Proxl1), leading to an arterial rather than lymphatic phenotype in endothelial cells (Kang et al. 2010, Srinivasan et al. 2010, Francois et al. 2011). COUP-TFII, in turn, can also suppress Notch signaling to result in vein rather than artery formation (You et al. 2005). Transforming growth factor β1 (TGFβ1) suppresses COUP-TFII expression in keratinocytes and fibroblasts leading to induction of collagen type VII (COL7A1) expression (Calonge et al. 2004) and in vascular progenitor cells to negatively regulate lymph vasculogenesis (Vittet et al. 2012). Whether COUP-TFII is regulated via Notch and TGFβ1 signaling has not yet been explored in cancer.

Amplification of Wnt/β-catenin signaling has been widely reported in cancer (Incassati et al. 2010). In normal tissues, β-catenin signaling is controlled through signals leading to its phosphorylation by a multiprotein destruction complex and subsequent degradation. In breast and other cancers, increased expression of Wnt ligands leads to maintenance of β-catenin activation by preventing its degradation (Incassati et al. 2010). β-Catenin signaling has many outcomes, such as normal mammary morphogenesis and ductal maturation; however, sustained activation, through a variety of mechanisms, leads to carcinogenesis (Incassati et al. 2010). ChIP assays demonstrated that β-catenin/TCF7L2 (T-cell factor 7-like 2 or transcription factor 7-like 2) binds the promoter of COUP-TFII to activate expression, resulting in suppression of adipocyte differentiation (Okamura et al. 2009). COUP-TFII is expressed in mouse liver and pancreatic β-cells and plays roles in the maintenance of glucose homeostasis and insulin sensitivity (Bardoux et al. 2005, Perilhou et al. 2008a). Boutant et al. (2012) also reported that β-catenin/TCF7L2 induces COUP-TFII expression in the pancreas and that COUP-TFII expression was necessary for normal β-cell function and glucose tolerance in mice. The influence of β-catenin signaling on COUP-TFII expression in cancer has yet to be examined.

**Role of COUP-TFII in cancer**

**Angiogenesis**

Many studies of COUP-TFII involve its regulation of the angiogenesis pathway. Under normal conditions, angiogenesis is not active after the time of vasculature development during embryogenesis. However, upon progression of a tumor’s growth, activation of angiogenesis leads to the formation of new blood vessels to support the tumor (Hanahan & Weinberg 2011). COUP-TFII is necessary during normal development for angiogenesis and lymphangiogenesis, as evidenced by the impaired vessel formation and embryonic lethality in COUP-TFII knockout mice (Pereira et al. 1999, Lin et al. 2010). The expression of many pro-angiogenic factors is modulated by COUP-TFII, including members of the vascular endothelial growth factor (VEGF) family and their receptors. VEGF induces angiogenesis and lymphangiogenesis by activating tyrosine kinase receptors and upregulates endothelial cell proliferation and migration (Hoeben et al. 2004). In a model of pancreatic islet tumorigenesis, ablation of COUP-TFII increased VEGF-R1 expression, impairing VEGFR2 signaling and reducing angiogenesis (Qin et al. 2010b). Metastasis to regional lymph nodes was reduced as a result, implying that COUP-TFII may have a pro-angiogenic, pro-metastatic role in pancreatic cancer (Qin et al. 2010b). Similarly, ablation of COUP-TFII decreased tumorigenesis in B16–F10 melanoma and Lewis lung carcinoma mouse xenografts and reduced tumorigenesis and metastasis in a spontaneous mouse mammary tumor model. These effects were attributed to a decrease in blood vessel density in COUP-TFII-deficient mice (Qin et al. 2010a).

In addition to regulating VEGF expression, COUP-TFII can also affect angiogenesis via regulation of Ang-1, through binding to an Sp1 site in the promoter region. The induction of Ang-1 is partially responsible for the effects of COUP-TFII, as overexpression of Ang-1 allowed for recovery of angiogenesis in COUP-TFII-deficient mice (Qin et al. 2010a).

Lymphangiogenesis can also contribute to metastasis by allowing the spread of tumor cells to lymph nodes (Achen et al. 2005, Tobler & Detmar 2006). COUP-TFII regulates tumor lymphangiogenesis via inducing expression of VEGF-C and neuropilin-2, a coreceptor for VEGF-C (Nagasaki et al. 2009, Lin et al. 2010). In a murine model of pancreatic islet tumorigenesis, COUP-TFII deletion resulted in impaired lymphangiogenesis.
and reduced metastasis (Qin et al. 2010b). Concordant with a role for COUP-TFI in lymphangiogenesis, Kang et al. (2010) reported that Notch suppresses COUP-TFI expression, along with Prox1, in human primary dermal lymphatic endothelial cells to signal for arterial rather than lymphatic differentiation. Suppression of COUP-TFI resulted in an increase in VEGF signaling by activating expression of VEGFR2, a VEGF receptor whose signaling can feed back to increase activation of Notch signaling (Kang et al. 2010).

COUP-TFI induction by 9cRA was also shown to promote network formation but not cell fusion in SKBR3 breast cancer cells, suggesting a role in the endothelial transdifferentiation pathway as a necessary part of vascular formation (Prahalad et al. 2010). Taken together, these data indicate that COUP-TFI may regulate angiogenesis and lymphangiogenesis, primarily through modulation of VEGF and its receptor in a cell context-dependent manner.

**Invasion and metastasis**

In addition to stimulation of angiogenesis, COUP-TFI may have other distinct roles in regulation of tumor growth and metastasis. Transfection with COUP-TFI in A549, H520, and H441 lung cancer cells and MDA-MB-231 breast cancer cells was reported to increase migration and invasion (Navab et al. 2004). Navab et al. (2004) found that COUP-TFI upregulated the expression of extracellular matrix-degrading proteinases matrix metalloproteinase 2 (MMP2) and urokinase-type plasminogen activator (uPA). MMP2 and uPA are known to play critical roles in cancer, particularly in angiogenesis and metastasis (Annecke et al. 2008). High levels of uPA are predictive of not only recurrence but also a favorable response to adjuvant chemotherapy in breast cancer patients (Harbeck et al. 2004). Interestingly, it has also been reported that uPA expression is dependent on Notch signaling in MDA-MB-231, MDA-MB-468, and HCC1143 breast cancer cells (Shimizu et al. 2011). COUP-TFI and MMP2 expression were also positively correlated in a breast tumor microarray (Litchfield et al. 2012), further indicating a potential relationship between COUP-TFI and extracellular matrix degradation. By contrast, COUP-TFI decreased cell motility when transfected into LY2 tamoxifen-resistant breast cancer cells, while having no significant effect on invasion (Riggs et al. 2006).

**Estrogen receptor and clinical outcome**

Nagasaki et al. (2009) demonstrated that COUP-TFI expression was correlated with ERα status and indices of poor clinical outcome (clinical stage, lymph node status, and histological grade) in human breast tumor samples, indicating that COUP-TFI may play a role in cancer progression. We also found that COUP-TFI and ERβ expression were correlated in a human breast tissue/tumor microarray, but instead noted an inverse relationship between COUP-TFI expression and tumor, node, and metastasis (TNM) classification (Litchfield et al. 2012). Similar findings were observed at the mRNA level by examining breast tumor mRNA transcriptomes in Oncomine (Litchfield et al. 2012). COUP-TFI expression was significantly higher in ERα+ breast cancer samples and significantly lower in metastatic samples (Litchfield et al. 2012). These findings indicate a function for COUP-TFI in inhibiting tumor progression. A positive correlation with ERβ is consistent with a previous report that siRNA knockdown of ERβ in MCF-7 breast cancer cells decreased COUP-TFI expression and treatment with estradiol increased the expression of COUP-TFI (Riggs et al. 2006). ERβ is a positive prognostic factor in breast tumors and is the target of endocrine-targeted cancer therapeutics such as the selective ER modulators (SERMs) tamoxifen and raloxifene (Jordan 2009). COUP-TFI, but not COUP-TFI, is reduced in tamoxifen-resistant human breast cancer cells, and re-expression of COUP-TFI can restore tamoxifen sensitivity (Riggs et al. 2006). As ERβ expression is important in keeping breast cancer cells responsive to treatment, the correlation of COUP-TFI and ERβ further demonstrates a beneficial role for COUP-TFI, highlighting its potential importance in maintaining differentiation and endocrine sensitivity.

In contrast to a role for COUP-TFI in maintaining antiestrogen sensitivity, Holbeck et al. (2010) reported that cancer cells in the NCI60 panel expressing low levels of COUP-TFI showed higher sensitivity to microtubule-targeting drugs vinblastine, colchicines, and taxol. These data demonstrate that both cell type-specific as well as drug-specific mechanisms may determine the role of COUP-TFI in influencing treatment response.

**Steroidogenesis**

COUP-TFI expression was reported to be high in aldosteroma, with an inverse correlation with adrenal steroidogenesis (Suzuki et al. 2000b). These data also indicated an inverse correlation between COUP-TFI expression and CYP17A1 expression, with COUP-TFI inhibiting CYP17A1 in aldosteroma (Suzuki et al. 2000b). COUP-TFI competed with SF-1 for binding to overlapping sites within the promoters of the Cyp17a1 (Bakke & Lund 1995, van den Driesche et al. 2012), Cyp11a1, and Star (STARD1) genes in rat Leydig cells and to suppress testosterone production (van den Driesche et al. 2012). Both COUP-TFI and COUP-TFI repressed angiotensin II-stimulated STARD1 (STAR) in
bovine adrenal glomerulosa cells in primary culture (Buholzer et al. 2005). COUP-TFII also competed with SF-1 for the human aromatase P450 promoter II in primary endometriotic stromal cells and suppressed aromatase expression (Zeitoun et al. 1999). Over-expression of SF-1 in primary endometriotic stromal cells outcompeted the normal protective effect of COUP-TF (whether COUP-TF-I or COUP-TFII involved was unclear as both were equally expressed at the mRNA level), resulting in high local aromatase expression in endometriosis (Zeitoun et al. 1999). COUP-TFII was reported to bind the S1 silencer region of the human aromatase gene and suppress transcription in MCF-7 cells (Yang et al. 2002). Indeed, the decreases in COUP-TFII, EARy, EAR-2, Snail, and Slug in breast cancer were suggested to increase aromatase expression (Chen et al. 2005). Thus, the downregulation of COUP-TFII expression that we observed in endocrine-resistant breast cancer cells (Riggs et al. 2006) would be expected to increase aromatase and thus increase local estrogen production. However, whether increased COUP-TFII suppresses local androgen or estrogen biosynthesis in breast tissue is unknown. Local conversion of adrenal androgens to estrogens by aromatase is the target of aromatase inhibitor (AI) therapy for postmenopausal women. However, there are androgen metabolites (e.g. 3β-adiol) that bypass aromatase, which activate ERα and ERβ and may play a role in AI resistance (Sikora et al. 2009, 2012). Overall, the literature supports a negative role for COUP-TFII in regulating steroid hormone synthesis and further studies addressing COUP-TFII regulation of aromatase gene expression in local estrogen production in breast (Bulun et al. 2012) and lung (Marquez-Garban et al. 2009) adenocarcinomas would be of merit.

Conclusions

The studies reviewed here indicate that COUP-TFII is regulated and is functionally active to regulate target gene transcription in a cell type-dependent manner. There is evidence that COUP-TFII may perform both pro- and anti-tumorigenic roles. COUP-TFII has been reported to increase angiogenesis and lymphangiogenesis, both increase and decrease tumor metastasis, lead to favorable and unfavorable therapeutic outcome in cancer therapy, and suppress steroidogenesis. Qin et al. (2010a) reported that COUP-TFII was not expressed in tumor cells, but rather was found in high concentrations in the surrounding blood vessels that support tumor growth and spread. This indicates a crucial point of consideration about the nature of COUP-TFII in cancer formation and progression: the function of COUP-TFII within cancer cells vs in the surrounding tumor microenvironment and other cell types. Tissue type is clearly an important determinant in deciphering the oncogenic or tumor-suppressive nature of COUP-TFII. Many studies published to date involve the regulation and role of COUP-TFII during development and in noncancerous disease states. The full applicability of these studies to our knowledge of the role of COUP-TFII in carcinogenesis and cancer progression remains to be seen. Future studies are necessary to elucidate the complex nature of this vital NR.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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