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Identification of a novel multiprotein complex in cargo sorting that preserves metabolic pathways in the liver

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CHAPTER 1

General introduction

1.0 COMMD family of proteins

The Copper Metabolism MURR1 Domain (COMMD) proteins constitute a family of proteins characterized by a carboxyl-terminal homology domain called the COMM domain (COMMD)¹. The COMMD proteins are ubiquitously expressed and are conserved throughout evolution from lower organisms to vertebrates¹. This strong conservation between species suggests that COMMD proteins have vital and unique functions. Although the amino-terminal region of COMMDs is conserved between species, the amino-terminal region differs between the members of the family (Figure 1).

A.

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COMMD1_MOUSE 10 20 30 40 50 60 70 80
COMMD2_MOUSE .....MAGDLEGGKSLGGLSGLAQ.....NAFHGHSGVTEELLHSQLYP-
COMMD3_MOUSE .....MLLDLSEEHKEHLAFLPQVDATAVVAEFGRIAVAEFLRRGSSNPKI-YEGAARKL-
COMMD4_MOUSE .....MELSESVORGIOTL...ADPGSFDNAFALLRAAFOSL-LDARADEAALDHP
COMMD5_MOUSE .....MRFRFCGDLDCPDWVLAELISTLAKISSVKLRLLCQVLLKELLG-QQID-YEKILKLTADA-
COMMD6_MOUSE .....APAPYLHHPDSSHSGRV5FLGSQPS...AEVTAVQALLKDLDRSTFRKLLKLVVGAALHGKD-CREAVQHLGASA-
COMMD7_MOUSE .....MGR LHCTQDPVPEAVRGMQQLNQLGAQQFSDLTEVLFHFLT EPKEVER-FLAQLSEFATSN-
COMMD8_MOUSE .....MEPEEGTLPWRLOKLPPEQGAGLLHKIIDGFCGRAYPAH-QDYH-...S-
COMMD9_MOUSE .....MAALTAEHFVALQSLKKA SSKDVVRQLCQESFSSCLDS-ESLLDKTCSSL-
COMMD10_MOUSE .....MAASAALI LPESPSMKAVPLINAIIDTGRFPRLLSRI LQKLHLKAESSF-SEEEEEKQAAAF-

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B.

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COMMD1_MOUSE 170 180 190 200 210 220 230
COMMD2_MOUSE .....NGLRGLSWRVDGKLSQSRHSTQIHSPVAILELFRGK.....NGQSEFLCLEDEVK-
COMMD3_MOUSE .....SMHSLSWRLDVQLASRSLRQQIKPAVTIKLHLDQNGD...HS--THFLQTPATLLHLVQQLLEQAL-
COMMD4_MOUSE .....HITDVSWRLEYSIKTQNLHKMYRPGYLVTLNVENNDS...QSYPEINFSOMMEDLQDLVGLKLDAS-
COMMD5_MOUSE .....NRLASVGRVBYTLLSSLLHSEEPMWHLQIQVPAAPG...TQAQPVSMSSLADKFOVLLAEKQAO-
COMMD6_MOUSE .....RVSNFRWRVDVAISTSAQSRSLQPSVLMQKLTDG...SAHRFEVPIAKFOELRY-
COMMD7_MOUSE .....GOLLIDFQWKLGMAVSSDCRSLSKYPYVAVMLKVADHSG...QVSSKSIEMTIPOFQNFYKQKELIA-
COMMD8_MOUSE .....NQLIDMEWRFG-VTSGSSELEKVGSI FLOKLVVKKG...KQTNELMYELTLPQFYFSLHEMERVR-
COMMD9_MOUSE .....QLQDFDWQLKLA LSSDKIATLQMLNLNLHLDWKEDD...KVKPYTVEMSKLEQLSLISSLEAAN-
COMMD10_MOUSE .....RLVLDLWRVDIKTSSDNISRMVAVPTCLLOMKIQEDP SLCGKPSI SAVTMELSKETLDTMDLGLGRIR-
COMMD10_MOUSE .....HKLETYGVQLNLQMAHSAQAKLQSPQAVLQGLGSKEDA...KNVEKVLVEFNHKLFDFFYKLETLQ-

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Figure 1. Sequence alignment of murine COMMD family of proteins.

A. An alignment of the N-terminal part of the mouse COMMD proteins. **B.** An alignment of the COMM domain of the mouse COMMD proteins. Jalview used for alignment and analysis. Entire alignment colored according to the hydrophobicity of the residues; red, most hydrophobic, blue, hydrophilic. Conservation then calculated with conservation threshold 20; all colored residues are above threshold.

COMMD6 is the only member whose amino-terminal region is not conserved between non-vertebrates and vertebrates². Vertebrate COMMD6 lacks an amino-terminal region and consists almost only of the COMM domain. This makes COMMD6 the most valuable member of the family to clarify the biological function of the COMM domain and thereby the protein family. The COMM domain is essential for the interaction with each other

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and with their interaction partners¹⁻³. The COMMDs have no enzymatic activities; therefore it is very likely that they act as scaffold proteins to tether other proteins into complexes⁴. Our current biological knowledge of this family comes mainly from studies of COMMD1, the family prototype^{1,5}.

1.1 COMMD1

COMMD1 (previously called MURR1) is a multifunctional protein, initially discovered as a protein to preserve copper homeostasis in mammals. Homozygous exon 2 deletion of *COMMD1* is linked to copper toxicosis in dogs⁵, a disorder characterized by hepatic copper accumulation. Almost at the same moment, COMMD1 was shown to act as an inhibitor of HIV-1 replication in lymphocytes by suppressing the activity of nuclear factor (NF)- κ B⁶. These initial studies already identified COMMD1 as a pleiotropic protein, a fact later supported by many other studies in which COMMD1 was correlated with processes like inflammation, hypoxia signaling^{7,8}, cancer^{8,9}, scavenging of free radicals¹⁰, trafficking of transmembrane proteins like the copper transporter proteins ATP7A/7B¹¹⁻¹³, epithelial sodium channel (ENaC)¹⁴, sodium–potassium–chloride cotransporter (NKCC1)¹⁵, cystic fibrosis transmembrane conductance regulator (CFTR)¹⁶ and the low-density lipoprotein receptor (LDLR)¹⁷. Some of the pathways will be discussed in more detail below.

1.2. COMMD1 in copper homeostasis.

COMMD1 has been discovered to be an import regulator in biliary copper excretion. Dogs (Bedlington terriers) carrying a homozygous loss-of-function mutation in *COMMD1* suffer from progressive copper accumulation in the liver due to impaired copper excretion into the bile⁵. Its role in copper homeostasis has been confirmed in a liver specific *Commdl* knockout mouse model¹⁸. However, in contrast to dogs, these mice only progressively

accumulate hepatic copper after a copper-rich diet challenge¹⁹. The interaction of COMMD1 with the copper-transporting P-type ATPase ATP7B, a gene product responsible for the human hepatic copper storage disorder, Wilson disease^{12,20,21}, further supports its role in copper homeostasis. Various cellular studies (described in chapter 2) showed that COMMD1 insufficiency affects the protein levels of ATP7B, and it has been hypothesized that COMMD1 is involved in directing ATP7B to particular vesicular compartment within a cell. Mislocalization of ATP7B likely results in enhanced proteolysis of ATP7B and may explain the reduced ATP7B levels observed in different COMMD1 deficient models^{18,21,22}. In line with the observation that COMMD1 is required for proper ATP7B levels, COMMD1 also interacts and regulates the protein levels of ATP7A^{12,23}. ATP7A is also a copper-transporting P-type ATPase protein and is highly homologous to ATP7B. Although the mechanism by which COMMD1 regulates ATP7B functioning has been an area of discussion (reviewed in chapter 2 of this thesis), the latest findings on COMMD1 have finally shed a light on the function of COMMD1 in copper homeostasis¹³. Here it has been shown that COMMD1 acts as an important component of the endosomal sorting machinery facilitating the trafficking of ATP7A, and it is very likely that COMMD1 regulates the function of ATP7B in hepatocytes in a similar manner¹³.

The trafficking of ATP7A/B is a complex and dynamic process, regulated by the intracellular copper content. Under low-copper conditions, the transporters reside in the trans-Golgi network (TGN), and upon copper excess they are mobilized to cytosolic endosomal vesicles²⁴. From these peripheral endosomal vesicles ATP7A/B reaches the plasma membrane to export copper out of the cell. In a recent study, Phillips-Krawczak and colleagues identified a new protein complex that regulates the endosomal sorting of ATP7A. This protein complex is called the CCC (COMMD/CCDC22/CCDC93) complex and consists of COMMD1, coiled-coil domain-containing protein 22 (CCDC22), coiled-coil domain-

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containing protein 93 (CCDC93) and C16orf62 (Figure 2). Mutations in *CCDC22* are correlated with X-linked intellectual disability in humans^{3,25,26}, and likely also cause aberrant copper homeostasis, as patients carrying the *CCDC22* c.49A>p.T17A mutation have increased serum copper and ceruloplasmin levels, although no signs of hepatic copper toxicity has been observed in these patients. However, fibroblast cells from these patients displayed abnormal distribution of ATP7A and the copper-dependent redistribution of ATP7A was also affected, as in the case of fibroblast cells with reduced levels of COMMD1¹³. The increased cellular copper content of fibroblast cells in which *CCDC22* has been silenced further indicates that *CCDC22* and *COMMD1* act together as a complex to preserve copper homeostasis by controlling the intracellular trafficking of ATP7A.

This study also identified a physical association between the CCC complex and retromer and WASH (**W**iskott-**A**ldrich syndrome protein and **S**CAR **H**omologue)¹³, both multiprotein complexes of the endosomal sorting machinery^{27,28}. Retromer is an evolutionarily conserved protein complex, consisting of VPS26, VPS29, and VPS35, which are recruited to the late endosomes through interaction with Rab7-GTP and sorting nexin complexes²⁹⁻³². Although retromer was originally identified in yeast as mediating retrograde trafficking of cargos to Golgi, later it was also shown to play a role in recycling many cell surface receptors back to plasma membrane³³, including ATP7A¹³. VPS35 deficiency results in decreased levels of ATP7A at the membrane during high copper exposure¹³. Furthermore, loss of retromer affects the total cellular levels of ATP7A due to disturbed endosomal sorting^{13,34}.

The WASH complex is composed of WASH1, FAM21, Strumpellin, SWIP, and CCDC53 and is recruited to endosomes through VPS35 via the interaction of FAM21^{27,28,35}. FAM21 is also required for the binding of the CCC complex with the WASH complex, which is essential for normal ATP7A recycling^{13,28}. WASH is a member of the WASP superfamily

of ARP2/3 F-actin nucleation promoting factors. These factors promotes branched F-actin nucleation at retromer-enriched endosomal subdomains^{36,37}. The activity of the WASH complex is required for the endosome-to-cell surface recycling of different receptors^{13,37,38}. Depletion of WASH results in enlarged and collapsed endosomes and lysosomes. Although these collapsed endosomes still contain segregated domains the recycling of WASH-dependent cargos, such as the EGFR, are markedly impaired^{27,37,39}.

Remarkably, all COMMD members can interact with each other¹ and have the ability to bind via the COMM domain to the CCC-component CCDC22³. However it remains unclear whether they all participate in the CCC-complex and share biological functions, as has been demonstrated for COMMD1, COMMD3 and COMMD9. These members of the COMMD family mediate sodium transport by altering trafficking of epithelial sodium channel (ENaC) and consequently its expression at the cell surface⁴⁰⁻⁴², but whether they act together needs to be elucidated.

1.3 COMMD1 in cholesterol homeostasis

A high level of circulating low-density lipoprotein (LDL) cholesterol is a major risk factor for coronary heart disease, and the LDL receptor (LDLR) is a central player in controlling levels of plasma cholesterol. LDLR is a transmembrane glycoprotein that has a multidomain structure, including an N-terminal ligand-binding region, an epidermal growth factor (EGF)-precursor homology region, a region containing O-linked sugars, a transmembrane domain and a C-terminal cytosolic domain⁴³. Synthesized LDLR is transported from the endoplasmatic reticulum to the Golgi, where glycosylation of the receptor takes place (Figure 2).

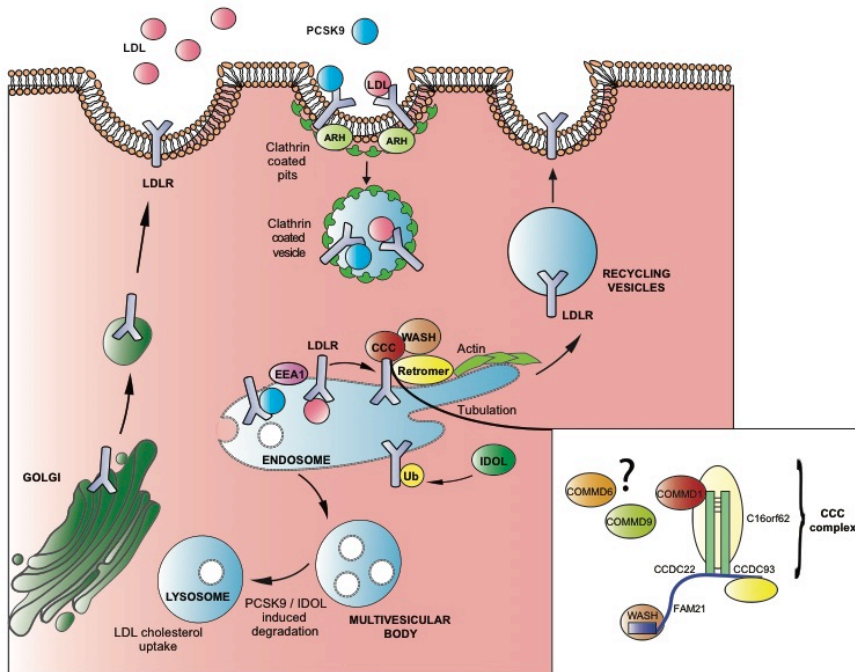


Figure 2. Simplified model of the LDLR recycling pathway.

LDLR directed from Golgi to cell surface. At cell surface LDL cholesterol (LDL) binds to LDLR and upon binding of the Autosomal Recessive Hypercholesterolemia protein (ARH) to the receptor's cytosolic tail, LDL-LDLR complex is internalized in clathrin-coated pits and sorted at endosomes. From the endosome LDLR can be recycled back to cell surface or directed to multivesicular bodies and subsequently to lysosomes for proteolysis. Lysosomal degradation of LDLR mediated by PCSK9- or IDOL. PCSK9 binds to extracellular domain of LDLR and directs LDLR to lysosomes. IDOL binds to cytoplasmic domain of LDLR and ubiquitinates receptor and itself. Through interaction of FAM21 with retromer component VPS35, WASH and CCC are recruited to endosomes. Subsequently, CCC and WASH form a protein complex with LDLR; WASH mediates branched actin networks on endosomes. These actin patches define regions into which LDLR is sorted back to the cell surface. Inset in right lower corner of figure depicts the organization of CCC (COMMD1, CCDC22, CCDC93 and C16orf62) in complex with WASH (partially adapted from¹³). It remains unclear whether other COMMD proteins participate in the CCC-complex to regulate the endosomal trafficking of ATP7B and LDLR. In this thesis we investigated the contribution of COMMD6 and COMMD9 to copper and cholesterol homeostasis, and to inflammation. (EEA1, Early endosome antigen 1; CCC,

COMMD1/CCDC22/CCDC93/C16orf62; WASH, WASH1, FAM21, strumpellin, KIAA1033 (SWIP), CCDC53; Ub, ubiquitin).

The mature glycosylated form of the receptor resides at the plasma membrane, where it clusters in coated pits, regions of the cell surface that are adapted for rapid internalization by endocytosis^{44,45}. Upon binding of LDL cholesterol (LDL-c) to the receptor at the cell surface the LDLR–lipoprotein complex is internalized through clathrin-coated pits into the vesicles⁴⁶, which fuse with early endosomes. LDL bound to the ligand-binding domain is released from the LDLR into the acidic environment of the sorting endosome. LDL-c is sorted to the late endosomes and eventually to the lysosomes, from which the cell takes up the cholesterol. LDLR either recycles back to the plasma membrane for reuse or is directed to the lysosomes for degradation. Degradation of LDLR can be mediated via two different pathways, which are controlled by Proprotein convertase subtilisin/kexin type 9 (PSCK9) or Inducible degrader of the low-density lipoprotein receptor (IDOL)^{47,48}. Although much is known about the intracellular trafficking route and the degradation pathway of LDLR, the mechanisms by which internalized LDLR is sorted at the endosomes either to the cell surface or to the lysosomes remain unclear. Recently we discovered that the CCC and WASH complexes are both essential for the normal endosomal sorting and function of LDLR (Figure 2). Inactivation mutations in *COMMD1*, *CCDC22* or *Strumpellin* result in hypercholesterolemia in mammals, including humans¹⁷. We found that COMMD1 interacts via the COMM domain with the cytoplasmic tail of LDLR. Furthermore, components of the WASH complex also bind to LDLR. We showed that loss of either COMMD1 or WASH impairs LDLR sorting, resulting in decreased LDLR levels at the cell membrane, and consequently reduced LDL uptake and eventually hypercholesterolemia. Although we were able to show that WASH-mediated F-actin polymerization on endosomes is essential for the endosomal trafficking of LDLR, the function of the CCC complex in endosomal LDLR trafficking is still unclear. The CCC complex might be involved in cargo recognition, and the

composition of the CCC complex could be important in determining which cargo is specifically sorted, as has been demonstrated for COMMD5 and COMMD9⁴⁹. Interestingly, among all COMMD family members that can associate with the CCC complex, only COMMD9 and its binding partner, COMMD5, have a substantial effect on Notch receptor trafficking, a fact which further indicates the specificity of COMMDs in cargo recognition⁴⁹.

1.4. COMMD proteins in inflammation

The NF- κ B family of proteins regulates the expression of genes involved in immunity and inflammation. Activation of NF- κ B results in recruitment and stimulation of various immune cells by inducing the transcription of proinflammatory molecules, such as cytokines. This process provides efficient protection of organisms against pathogens and injuries. However, it is essential that NF- κ B does not stay permanently active as this can lead to chronic inflammation. Chronic inflammation participates in the development and pathogenesis of many diseases, such as atherosclerosis, diabetes and cancer⁵⁰⁻⁵². Therefore, to maintain a healthy state, NF- κ B activity needs to be tightly regulated. This is provided by several factors that can negatively regulate NF- κ B signaling^{53,54}; one of these factors is the presence of COMMD proteins.

The NF- κ B family consists of five members, including RELA (p65), RELB, c-REL, p50/p105 (NF- κ B1), and p52/p100 (NF- κ B2). The dimer combination of NF- κ B members determines whether NF- κ B acts as a transcriptional activator or a repressor. Canonical NF- κ B activity is mediated primarily by RELA/p50 complex. Under basal conditions this complex interacts with the inhibitory I κ B proteins that mask their nuclear localization sequence, keeping NF- κ B inactive in the cytosol. The activation of NF- κ B requires degradation of I κ B triggered by the I κ B kinase complex, which acts as a negative feedback loop in NF- κ B signaling. In 2005 Burstein *et al* reported that COMMD1 is able to interact

with all five subunits of NF- κ B, whereas the other COMMDs interact with particular NF- κ B subunits¹. When overexpressed, all COMMD members can inhibit TNF-induced NF- κ B activity *in vitro*, yet to different degrees^{1,2}. Although all ten COMMD proteins interact with NF- κ B, so far a detailed mechanism has been described only for COMMD1. COMMD1 inhibits NF- κ B by promoting the ubiquitination and subsequently the proteasomal degradation of RELA bound to chromatin^{4,55} by acting as a coactivating factor for a Cullin2-RING ligase that ubiquitinates RELA. Cullin-RING ligases (CRLs) are ubiquitin ligase multiprotein complexes containing members of the Cullin family (Cul1-7) and acting as a scaffold protein within CRLs⁵⁶. CRLs regulate diverse cellular processes, including cell cycle progression, DNA repair, and many signal transduction pathways, including NF- κ B⁵⁷. Like CRL-mediated RELA degradation⁴, the degradation of I κ B also depends on CRL (Cullin1-RING ligase)⁵⁸. Besides COMMD1, other COMMD proteins also interact with Cullin proteins but have particular preferences for certain Cullins. For example, only COMMD8 and COMMD10 interact with Cullin1⁵⁹, and it is likely that specific Cullin-COMMD complexes regulate specific steps of the NF- κ B signaling pathway, as has been demonstrated for COMMD8. COMMD8 in conjunction with CCDC22 and Cullin1 promotes the protein degradation of I κ B α . Loss of COMMD8, as well as mutation in CCDC22, affects I κ B α degradation and consequently reduces NF- κ B transcriptional activation³. The effect of COMMD8 on I κ B α degradation seems to be COMMD specific, as loss of either COMMD1 or COMMD10 does not affect TNF-induced I κ B α degradation. These data suggest the existence of different COMMD-CCDC22 complexes involved in NF- κ B signaling; as myeloid COMMD1 plays an important role in suppressing inflammation in different disease models^{60,61} it is therefore of great interest to assess the function of other COMMD proteins in inflammation.

1.4. COMMD proteins in cancer

During the last ten years several COMMD proteins have been suggested to play a role in cancer progression. COMMD1 is known to affect tumor cell behavior and survival^{8,9,62,63} but the mechanism involved is still inadequately defined. One of the suggested mechanisms of COMMD1 in mediating the behavior of cancer involves its inhibitory action on the activity of Hypoxia-inducible factor 1 (HIF-1) and Nuclear Factor Kappa B (NF- κ B)^{8,63}. HIF-1 and NF- κ B are transcription factors and both have a significant role in tumor behavior and clinical outcome⁶⁴⁻⁶⁶.

A variety of mechanisms, including local hypoxia within rapidly growing solid tumors, are thought to lead to HIF activation in cancer. HIF controls energy metabolism, angiogenesis and tumor growth⁶⁷. Overexpression of HIF-1 is positively correlated with more aggressive tumor phenotypes, neovascularization, the formation of metastasis, poor prognosis, treatment resistance and increased tumor growth (reviewed in^{67,68}). Studies have shown that therapeutics affecting HIF regulation or its activity decrease HIF-1-mediated angiogenesis, cancer metabolic activity and metastatic niche formation. COMMD1 silencing leads to increased activation of HIF-mediated transcription. In particular, COMMD1 impairs HIF-1 α / β dimerization and binding of HIF-1 to the DNA⁸ and promotes the proteolysis of HIF-1 α ⁶⁹.

A number of human cancers have constitutive NF- κ B activity due to the inflammatory microenvironment and various oncogenic mutations. NF- κ B activity promotes tumor proliferation, abolishes apoptosis and stimulates angiogenesis (reviewed in⁵²). Suppression of NF- κ B in myeloid cells or tumor cells can lead to tumor relapse; this makes the NF- κ B pathway a favorable target for treating cancer⁵². All COMMD proteins can negatively regulate NF- κ B signaling *in vitro* (reviewed in⁷⁰), and interestingly, decreased levels of COMMD1 have been seen in many cancer types, including seminoma^{71,72},

pancreatic cancer⁷³ and ovarian cancer^{8,74}. Recently it was shown that COMMD1 is also downregulated during enrichment for stemness in head and neck squamous-cell carcinoma cells (HNSCC). In these cells COMMD1 is negatively regulated by microRNA miR-205, and silencing of COMMD1 in HNSCC cells promotes tumorigenesis and tumor growth *in vivo*. Furthermore, tumors derived from COMMD1-knockdown cells have increased NF- κ B activity⁶³.

Under normal physiological conditions COMMD1 is predominantly localized in the cytoplasm of most cell types^{13,75,76}, but an important role for COMMD1 in the nucleus has also been established^{13,75,76}. Despite the lack of a nuclear localization signal in COMMD1, its nuclear levels can be actively regulated via a nuclear export signal (NES) in an exportin 1 (CRM1)-dependent manner. Disruption of the NES in COMMD1 results in elevated nuclear COMMD1 levels accompanied by increased repression of the transcriptional activity of NF- κ B and HIF-1⁷⁶. Additionally, it was suggested that nuclear COMMD1 participates in cellular response to DNA damage. In 2012 COMMD1 was identified as a new potential DNA damage response protein, as it was shown to interact with nuclear BRCT domain-containing proteins: Breast Cancer 1 Early Onset (BRCA1), BRCA1-associated RING domain protein 1 (BARD1) and checkpoint kinase 2 (Chk2)⁶². Ablation of COMMD1 results in increased sensitivity to DNA-damaging agents in several cell lines^{22,62}. The exact mechanism underlying this effect, and whether COMMD1 expression levels are associated with the response to platinum-based therapy in cancer patients, remains unclear and needs to be investigated.

Several other COMMD proteins have been reported to affect proliferation, cell cycle and tumor progression⁷⁷⁻⁷⁹. COMMD5 and COMMD7 have been associated with regulation of cell proliferation and cell cycles^{78,79}. COMMD5 controls cell growth and differentiation, which are associated with increased levels of p21^{78,80}. Additionally, COMMD5 plays a role in

kidney repair after injury by inducing p21 expression and its effect on renal cell migration and TGF- β secretion⁸¹. Using cellular models, it was shown that COMMD7 contributes to hepatocellular progression by reducing cell apoptosis and overcoming cell cycle arrest⁷⁹. So far it seems that different COMMD proteins can regulate distinct pathways in cancer cells, and it is of great interest to elucidate the pertinent mechanisms and their role in cancer.

1.5 Aims and Scope of thesis

It has been well established that scaffold proteins are crucial regulators of a diverse array of biological processes, by tethering other proteins into complexes⁸². The aim of this thesis is to better understand the function of the relatively new family of scaffold proteins called the COMMD proteins. COMMD1, the prototype of this family, has been associated with numerous diseases such as hepatic copper toxicity syndrome, hypercholesterolemia and cancer, but the biological role of the other members remains largely unknown. Increasing our fundamental knowledge of how this family of proteins fine-tunes cellular processes can lead to new opportunities to develop therapies. **Chapter 2** gives an overview of the current knowledge on the function of COMMD1 in copper homeostasis, and in **chapter 3** we studied the role of COMMD1 in cisplatin sensitivity in ovarian cancer. In **chapters 4, 5 and 6** we examine the contribution of other COMMD proteins, in particular COMMD6 and COMMD9, to copper and cholesterol homeostasis and liver inflammation.

Copper is a crucial cofactor in the activity of an array of enzymes involved in numerous critical biological processes. Although copper is vital for all living organisms, in excess it can have harmful effects on physiological functions. This is clearly illustrated in several hereditary forms of copper toxicity in humans and animals. A deleterious mutation in *COMMD1* has been found to be associated with copper toxicosis in dogs⁵, and the role of COMMD1 in copper homeostasis was confirmed in an elegant mouse model¹⁸. Yet, the

mechanism by which COMMD1 regulates copper homeostasis is still unknown. **Chapter 2** provides an overview of the current knowledge of the role of COMMD1 in copper homeostasis.

HIF and NF- κ B have been reported to participate in tumor growth and invasion⁶⁴⁻⁶⁶. The inhibitory role of COMMD1 in these pathways^{8,63} suggests that COMMD1 might also be a crucial factor in cancer. This notion is supported by various studies showing a correlation between COMMD1 expression and the survival of patients with different types of cancer^{8,9,61,63}. Furthermore, *in vitro* studies revealed that COMMD1 influences tumor behavior, including sensitizing cancer cell lines to cisplatin^{22,62}; however, the mechanism and its role in platinum sensitivity in cancer have yet to be established. **In chapter 3** we assessed the function of COMMD1 in cisplatin sensitivity in ovarian cancer cells and the relation between COMMD1 expression and response to platinum-based therapy in advanced stage high-grade serous ovarian cancer (HGSOC) patients.

The COMMD family of proteins consists of 10 members, but except for COMMD1, the biological function of the other members has not been adequately studied. We have demonstrated that COMMD1 deficiency in mammals causes hypercholesterolemia and hepatic copper toxicosis^{17,18}. Furthermore, COMMD1 controls inflammation by suppressing NF- κ B activity *in vitro* and *in vivo*^{1-3,60,61,70,83}. The observations that all COMMD proteins can interact with each other^{2,3,49} and suppress NF- κ B activity *in vitro*^{1,2} suggest that COMMD proteins most likely act together; however, the composition of these multi-COMMD complexes and their contribution to the aforementioned cellular processes remain unclear. In **chapters 4, 5 and 6**, using innovative mouse models we elucidated the role of other COMMD proteins (COMMD6 and COMMD9) in cholesterol and copper homeostasis and in inflammation. We used a combination of molecular and biochemical analysis to uncover the composition of the COMMD-associated protein complexes.

In **chapter 4** we used a liver specific *Commf6* knockout mouse model to assess the effect of COMM6 deficiency on cholesterol and copper homeostasis. Furthermore, we determined the contribution of COMMD6 to the functions of other COMMD proteins.

Studying the biology of COMMD proteins in mice is limited because deletion of individual *Commf* genes results in embryonic lethality^{7,49,84,85}. To bypass the lethality caused by *Commf6* ablation but still be able to study the role of COMMD6 at the organismal level we generated hypomorphic COMMD6 mice (**chapter 5**); these mice have lower than normal amounts of endogenous COMMD6. We studied the effect of this dramatic reduction in COMMD6 levels on different physiological processes.

In **chapter 6** we assessed the biological functions of myeloid and hepatocyte COMMD9, using conditional *Commf9* knockout mice. Prior studies demonstrated a repressive function for myeloid COMMD1 in inflammation^{60,61}; here we determined whether COMMD9 in the myeloid lineages also controls the inflammatory response. In addition, we investigated the effect of hepatic COMMD9 deficiency on clearing circulating cholesterol. Finally, in **chapter 7** we summarized and discussed the major findings of this thesis and gave directions for future research.

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