

Role of the human cytochrome b561 family in iron metabolism and tumors (Review)

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Abstract. The human cytochrome b561 (hCytb561) family consists of electron transfer transmembrane proteins characterized by six conserved α -helical transmembrane domains and two β -type heme cofactors. These proteins contribute to the regulation of iron metabolism and numerous different physiological and pathological processes by recycling ascorbic acid and maintaining iron reductase activity. Key members of this family include cytochrome b561 (CYB561), duodenal CYB561 (Dcytb), lysosomal CYB561 (LCytb), stromal cell-derived receptor 2 (SDR2) and 101F6, which are widely expressed in human tissues and participate in the pathogenesis of several diseases and tumors. They are associated with the promotion or inhibition of tumor growth and progression in various malignancies and are potential therapeutic targets for malignant tumors. The present review summarizes the existing literature regarding the structure of the Cytb561 family, the basic functional characteristics of hCytb561 family members, and the roles of the CYB561, Dcytb, LCytb, SDR2 and 101F6 in various diseases and tumors.

Contents

1. Introduction
2. Iron metabolism, ASC and tumors
3. Basic characteristics of the Cytb561 family
4. Expression and function of the hCytb561 family
5. Summary

1. Introduction

The B-type cytochrome family comprises electron transport proteins containing heme. The redox-active center of these proteins is iron protoporphyrin IX, which can be non-covalently bound to other protein matrices. Cytochrome b561 (Cytb561) is a member of the B-type cytochrome family, and is a transmembrane protein containing two heme-b subunits embedded within the membrane. It has a maximum absorbance wavelength in the redox absorption spectrum of ~561 nm, which is reflected in its name. The Cytb561 protein consists of 200-300 amino acids, approximately half of which are embedded within the membrane bilayer. Cytb561 can transmit electrons across chromaffin granule membranes (1,2) and facilitate transmembrane electron transfer (3).

The Cytb561 family is named after the specific cytochrome b561 (CYB561), which was identified and named in bovine chromaffin granule membranes in 1971 by Flatmark *et al* (4). Based on an analysis of the CYB561 gene sequence from chromaffin granules of the bovine adrenal gland, it was discovered that this protein family exists in various organs and cells across multiple species, of animals, including humans (5), mice (6), *Drosophila melanogaster* (7), *Anopheles gambiae* (8), *Caenorhabditis elegans* (9) and planarian species (10), and plants such as *Arabidopsis thaliana* (11) and cultivated rice (12). Cytb561, unique to eukaryotes, exhibits a high degree of conservation, implying similar structures and functions across different species (13). Mammalian Cytb561 proteins and predicted plant Cytb561 proteins are highly hydrophobic and can transfer electrons from the cytoplasmic side of the cell membrane to the extracellular space or intracellular vesicles. They have important roles in various physiological processes, including iron absorption, cellular defense, nitrate reduction and signal transduction. Through multiple sequence alignment, Cytb561 family members from various sources have been categorized into seven categories (14): Animals/neuroendocrine, plants, insects, fungi, animals/tumor suppression factor (TSF), plants containing a DoH domain, and stromal cell-derived receptor 2 (SDR2). In 1974, Silsand and Flatmark (15) purified Cytb561 from bovine chromaffin granules; however, other natural Cytb561 proteins have not been purified due to their low abundance in natural sources. Using the Basic Local Alignment Search Tool, six members of the human

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Cytb561 (hCytb561) family (16) have been identified: CYB561 encoded by CYB561A1; duodenal CYB561 (Dcytb) encoded by CYB561A2 (17); lysosomal CYB561 (LCytb) encoded by CYB561A3; SDR2 encoded by ferric chelate reductase 1 (FRRS1); 101F6/human TSF encoded by CYB561D2 (14); and 101F6 analogs (16). To date, the most extensively studied members of this family, which are associated with tumors, are CYB561, Dcytb, LCytb, SDR2 and 101F6.

In the present article, the structures of the Cytb561 family, basic functional characteristics of hCytb561 family members, and the roles of the five key family members in various diseases and tumors are reviewed.

2. Iron metabolism, ASC and tumors

Iron metabolism in the human body is a tightly regulated system, involving iron absorption, transport and distribution, the formation of various important functional ferritin molecules, storage and excretion (18). Iron is absorbed into the bloodstream via active transport by mucosal cells in the duodenum and upper segment of the jejunum. In the bloodstream, ferric ions (Fe^{3+}) are bound and transported by transferrin. After entering cells through transferrin receptor 1 (TFRC), these ions are reduced and released into the cytoplasmic labile iron pool (LIP), and excess iron is stored in ferritin (19). TFRC is an iron import protein that is post-transcriptionally regulated by iron levels; it contributes to iron absorption and is the main means of iron uptake in proliferating cells (20). Ferritin is an iron-binding protein that primarily functions to store and stabilize iron, and also acts as an iron oxidase, converting ferrous ions (Fe^{2+}) to Fe^{3+} during iron ion internalization and storage (21). Divalent metal transporter 1 (DMT1) is a proton-coupled transmembrane metal ion transporter in mammals, which mediates iron absorption in the small intestine and facilitates iron transport from the endosome (22). Fe^{2+} transported from the endosome by DMT1 contributes to the LIP, which is involved in cellular metabolism (23). Iron absorption is regulated by multifactorial feedback, and the synthesis of ferritin, transferrin and its receptor TFRC is regulated by iron levels in the body.

Ascorbic acid (ASC) acts as a cofactor for numerous important enzymes involved in mammalian metabolism (24), including dopamine β -hydroxylase in the vesicles of catecholamine storage granules (25) and peptide-amide monooxygenase in neuropeptide storage granules (24). The regeneration of ASC in neuropeptide storage vesicles is necessary to sustain amidated peptide biosynthesis. The cyclical regeneration of ASC contributes to cell physiology and serves as a key regulator of iron metabolism (26). This vitamin affects iron metabolism by promoting the intestinal absorption of non-heme iron (27), increasing transferrin-dependent iron uptake, and promoting the ASC/dehydroascorbic acid (DHA) cycle at the plasma membrane to increase the uptake of non-transferrin-bound iron (28-30). It also promotes ferritin synthesis by increasing or maintaining the level of iron regulatory protein 1 in its non-iron-bound form to promote cytoplasmic aconitase reactivity (31,32). Additionally, ASC regulates iron metabolism by inhibiting the autophagy of ferritin (33), inhibiting lysosomal ferritin degradation (33,34) and regulating cell iron efflux (30,35).

Iron is one of the most important trace elements in mammals due to its important role in cell replication, metabolism and growth (36). Iron also contributes to the generation of free radicals via oxidation and reduction, such as in the Fenton reaction (37), the destruction of lipids and proteins, DNA base modification, DNA strand breakage and other mutations associated with oxidative DNA damage (38), as well as the occurrence and development of cancer (39). To sustain high intracellular iron levels and promote the function of iron-dependent proteins, malignant tumor cells modulate the expression or activity of various iron-related proteins. For example, elevated plasma ferritin levels are associated with advanced clinical stages and poor prognosis in patients with hepatocellular carcinoma (40) and hepatobiliary cancer (41). DMT1 is highly expressed in colorectal cancer, and inhibiting its activity can restrict tumor growth by inhibiting the Janus kinase-signal transducer and activator of transcription 3 signaling pathway (42). Increased serum concentrations of heavy-chain ferritin (FTH) in patients with melanoma are associated with increased numbers of circulating CD4^+ CD25^+ regulatory T cells, which contribute to the suppression of antitumor immune function (43). Moreover, the proliferation of CD8^+ T cells, critical in the antitumor immune response, requires iron stored in FTH (44). As highlighted in a previous review, disrupted iron homeostasis can be observed at all stages of cancer development (45). Various iron metabolism-related proteins have been demonstrated to be involved in the initiation, proliferation and metastasis of malignant tumors (46). The expression levels of certain genes associated with iron metabolism have been indicated to be powerful indicators of tumor prognosis. For example, upregulated TFRC expression in breast cancer (20,47) and downregulated ferritin (48) or ferroportin expression (49) are associated with a poor prognosis. The regulation of iron homeostasis, by iron depletion and the targeting of iron metabolism, has shown strong and extensive antitumor effects, suggesting that iron metabolism is a potential target for cancer therapy (50). As the hCytb561 family plays an important role in iron metabolism and cancer, it is expected to become a key target for the treatment of malignant tumors.

3. Basic characteristics of the Cytb561 family

Basic structure. The Cytb561 family of transmembrane proteins typically contain six transmembrane α -helix domains (51-53). Sequence alignment indicates that all retrieved sequences exhibit four completely conserved sequences located in the second to fifth transmembrane segments (14). Four consecutive central helices form the core domain of CYB561 (Fig. 1). The core of the four helices comprises two pairs of histidine (His) residues (14,51); the first and third residues are located on the cytoplasmic side, while the second and fourth are located at the boundary region of the outer (or intravesicular) hydrophilic ring and transmembrane helix (54-56). The CYB561 core domain, via its four His residues, coordinates to heme-b groups on both sides of the membrane (57-59). The core domain facilitates intramolecular electron transfer by accepting electrons from ASC (60). It is structurally similar to other redox domains, such as dopamine β -monooxygenase redox domains and the dopamine β -monooxygenase N-terminal (DOMON) domain (24),

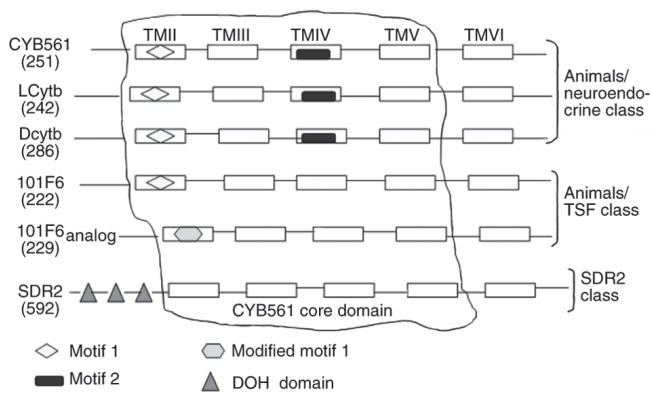


Figure 1. Location of the protein domain for human cytochrome b561 family members and their classifications. The number in parentheses under the name of each family member is the number of amino acids. TMII-VI, transmembrane domain II-VI; CYB561, cytochrome b561; DCytb, duodenal CYB561; LCytb, lysosomal CYB561; SDR2, stromal cell-derived receptor 2; TSF, tumor suppression factor.

and can be a component of other proteins. The basic characteristics of hCytb561 family members, including their genomic locations and sequence sizes, are shown in Table I. They are divided into different categories according to their specific motifs (Fig. 1). CYB561, DCytb and LCytb have been assigned to the animals/neuroendocrine class due to presence of motif 1 [FN(X)HP(X)₂M(X)₂G(X)₅G(X)ALLVYR] and motif 2 [YSLHSW(X)G] in their core structures (15). 101F6 and its analogs have been classified in the animals/TSF class due to the presence of modified motif 1 [LFSWHP(X)₂M(X)₃F(X)₃M(X)EAIL(X)SP(X)₂SS] in their structures. SDR2 belongs to the SDR2 class due to the presence of three DOH domains or a DOMON domain (14), which is homologous to a domain found in dopamine-β-hydroxylase (61). All family members have transmembrane electron transfer activity or contain at least two heme groups that may contribute to the integrity or binding of the transmembrane protein structure (56). The double-electrode voltage-clamp technique has demonstrated the ability of SDR2 to conduct electric current (62).

Human CYB561 is estimated to lack the first 22 amino acids at the N-terminal of the cytoplasmic side predicted for the bovine cell sequence. Srivastava *et al* (63) hypothesized that the gene product of human CYB561 contains five transmembrane helical structures. The crystal structure of *Arabidopsis thaliana* Cytb561 suggests that this protein may function as a dimer (13). Additionally, two highly conserved amino acids, Lys-81 and His-106, have been demonstrated to be important in substrate recognition and catalysis (64). To illustrate the structures of hCytb561 family members, data on their 3-dimensional structures have been obtained from the UniProt (Universal Protein Resource) website (<https://www.uniprot.org/uniprotkb>) and are presented in Fig. 2.

Basic function. The Cytb561 family uses ASC, an extracapsular electron donor, on one side of the membrane to provide electrons, catalyze transmembrane electron transport, and reduce monodehydroascorbate (MDA), a partially oxidized form of ASC that acts as an intracapsular electron receptor, thereby facilitating the regeneration of ASC (64,65). Motif 2 was originally thought to be an MDA free-radical-binding

sequence (60). The reduced expression of the CYB561 homologue in *Drosophila* memory mutant (nemy) has been shown to lead to defective memory retention, confirming the role of Cytb561 in memory retention and its influence on neural function (66). In addition, the role of Cytb561 in ASC regeneration has been confirmed by the fact that ASC in erythrocytes can reduce extracellular MDA (67,68). ASC has been confirmed to function as an electron donor for mouse DCytb (67). Furthermore, B-type cytochromes with biophysical properties similar to those of Cytb561 from bovine chromaffin granules have been reported to reduce ASC in the cell membranes of certain plants (69,70).

In vitro, all Cytb561 members exhibit Fe³⁺ reductase activity (71). DCytb in the cell membranes of mouse duodenal epithelial cells has been shown to reduce intestinal Fe³⁺ and thereby facilitate the uptake of iron, which ameliorates hypoxia and iron deficiency (17). In addition, the increased expression of DCytb in mice with total iron overload (hemo-chromatosis) promotes iron uptake (72). The transmembrane Fe (3+) reductase activity of CYB561 has been demonstrated in ASC-loaded protein liposomes (57). Furthermore, DCytb uses cytoplasmic ASC as an electron donor to reduce extracellular Fe³⁺ to Fe²⁺ in rabbit peritoneal neutrophils (73). Three members of the Cytb561 family, namely mouse Cytb561 and mouse and *Drosophila* SDR2, have been found to have iron reductase activity (71). These findings indicate that when free Fe³⁺ and ASC are in the same membrane compartment, Fe³⁺ easily obtains electrons from ASC and is reduced to Fe²⁺, which can generate free radicals via the Fenton reaction. However, when Fe³⁺, ASC and Cytb561 are separated, the ASC-mediated reduction of Fe³⁺ may be moderated. Whether Cytb561 family members reduce Fe³⁺ or MDA *in vivo* is likely to depend on the availability of the substrate and the biological environment.

4. Expression and function of the hCytb561 family

CYB561. First identified in chromaffin vesicles that synthesize catecholamines, CYB561 was later confirmed to be present in neuroendocrine tissues (74). RNA sequencing has shown that CYB561 is widely expressed in eight systems (locomotion, digestive, respiratory, circulatory, urinary, reproductive, endocrine and nervous systems) of the normal human body. According to gene expression data from the Bgee database (<https://bgee.org>), the CYB561 gene is expressed in the adrenal gland and 223 other tissues. Pathologically, CYB561 is expressed in HeLa cervical cancer cells and melanoma cells (74). In addition, its mRNA levels are significantly upregulated in SW480 colon cancer, HL-60 T-cell lymphoma and K-562 chronic myeloid leukemia cells but not in Burkitt's lymphoma (75). Under physiological conditions, CYB561 in natural chromaffin granules, vesicles and recombinant membrane systems exhibits 'electron shuttle' activity between ASC and membrane permeable ferricyanide or MDA (57,76), which is associated with cell metabolism and mitochondrial activation (77) (Fig. 3), and affects heart rate and blood pressure through regulation of the adrenaline pathway (78).

Pathogenic homozygous mutations in the CYB561 gene have been reported to lead to ASC deficiency in catecholamine-secreting vesicles and functional dopamine

Table I. Basic characteristics of the hCytb561 family.

Characteristics	CYB561	Dcytb	LCytb	SDR2	101F6
Gene name	CYB561A1/ FRRS2	CYBRD1/ CYB561A2	CYB561A3	FRRS1	CYB561D2
Genomic location	17q23.3	2q31.1	11q12.2	1p21.2	3p21.31
Sequence size, bp	14,336	35,897	13,539	62,666	17,353
Amino acids, n	251	286/174	242	592	222
Domain	19-220 CYB561	15-220/1-174 (isomorph) CYB561	12-219 CYB561	13-179 Reelin; 216-331 DOMON; 335-534 CYB561	14-217 CYB561
Subcellular location	CV, SV, CGM	CM, ACM	LEM, LM	CM	CV, ERM
Highest tissue expression	Brain, adrenal gland	Brush border membrane of duodenum	Adrenal gland	Liver, kidney	Pancreas, brain

hCytb561, human Cytb561; Cytb561/CYB561, cytochrome b561; Dcytb, duodenal CYB561; LCytb, lysosomal CYB561; SDR2, stromal cell-derived receptor 2; FRRS1/2, ferric chelate reductase 1/2; CYBRD1, cytochrome b reductase 1; DOMON, dopamine b-monooxygenase N-terminal; CV, cytoplasmic vesicle; SV, secretory vesicle; CGM, chromaffin granule membrane; CM, cell membrane; ACM, apical cell membrane; LEM, late endosome membrane; LM, lysosome membrane; ERM, endoplasmic reticulum membrane.

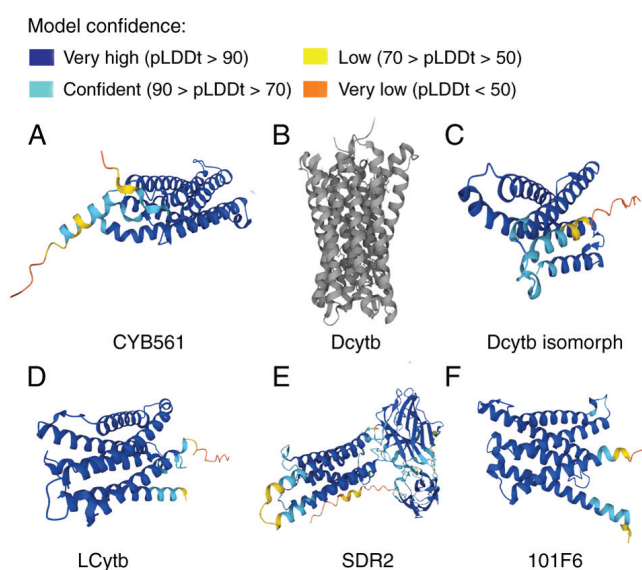


Figure 2. Structure of human Cytb561 family members. Protein structures of (A) CYB561, (B) Dcytb, (C) Dcytb isomorph, (D) LCytb, (E) SDR2 and (F) 101F6. The structures are from the UniProt website (<https://www.uniprot.org/uniprotkb>). The pLDDT is a per-residue confidence score generated by the AlphaFold prediction tool, which ranges between 0 and 100. The higher the value, the more confident the model is in predicting that residue. Some regions with low pLDDT may be unstructured in isolation. Due to the lack of stable structural features in these regions, the accuracy of model predictions is low. Cytb561/CYB561, cytochrome b561; Dcytb, duodenal CYB561; LCytb, lysosomal CYB561; SDR2, stromal cell-derived receptor 2; UniProt, Universal Protein Resource; pLDDT, Predicted Local Distance Difference Test.

b-hydroxylase deficiency, resulting in orthostatic hypotension syndrome (78). In CYB561^{-/-} mice, it was found that the concentration of norepinephrine in whole brain homogenate and adrenal glands was reduced compared with that in wild-type mice (78). CYB561 is also involved in the

progression of certain tumors and influences their prognosis. For example, a low expression level of CYB561 mRNA was found to be associated with a poor prognosis in patients with ovarian cancer, suggesting that CYB561 may be a single-gene prognostic biomarker (79). In addition, in another study CYB561 was demonstrated to promote the growth and metastatic potential of castration-resistant neuroendocrine prostate cancer (80). Our previous study found that CYB561 expression was upregulated in breast cancer; associated with human epidermal growth factor receptor 2 (HER2), immune cells, histological grade and molecular subtypes; and associated with the poor prognosis of patients (81), which is consistent with other results reported in the literature (82). Moreover, our previous study demonstrated that CYB561 promotes the proliferation, migration and invasion of breast cancer cells and inhibits apoptosis (83). The knockdown of CYB561 changes the Fe²⁺ and total iron content of cancer cells and the expression of the iron absorption and transport-related proteins TFRC and DMT1, indicating that it is involved in the iron metabolism of breast cancer cells (83). In addition, recent literature shows that CYB561 plays a role in promoting the proliferation of HER2-positive breast cancer cells by inhibiting the degradation of H2A histone family member Y (84).

Dcytb/cytochrome b reductase 1 (CYBRD1)/CYB561A2. Dcytb is a plasma membrane protein with 40-50% homology to CYB561 (17). The predicted ASC- and DHA-binding motifs in the Cytb561 family are highly conserved in Dcytb (17) (Fig. 3). The gene encoding Dcytb is widely expressed in human tissues and highly expressed in the duodenal brush border membrane. Dcytb serves as the plasma membrane iron reductase of mammals, facilitating the absorption of dietary iron at the brush edge of duodenal cells by reducing Fe³⁺ to Fe²⁺ and cooperating with DMT1 to transport Fe²⁺ to cells in the small intestine (21) (Fig. 3). It also participates in the regeneration of extracellular ASC (85) by reducing extracellular MDA (86),

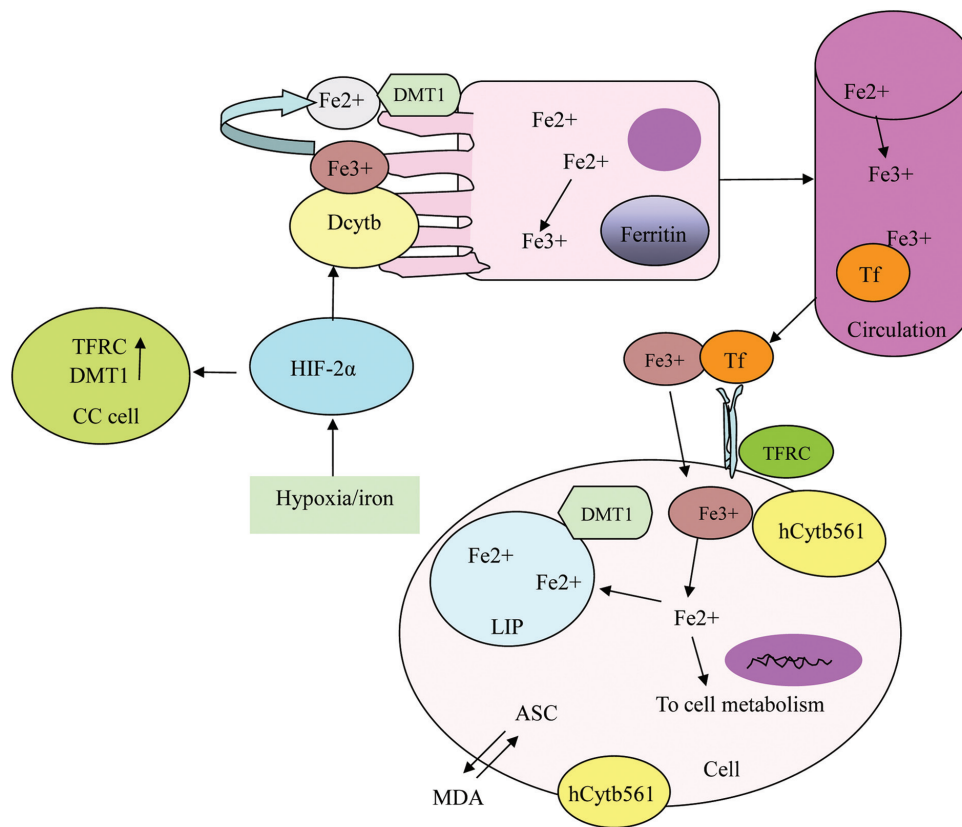


Figure 3. Function of the hCyb561 family in iron metabolism and the possible mechanism of Dcytb. All hCyb561 family members have iron reductase activity and participate in the regeneration cycle of ASC and the Fe redox reaction. Dcytb participates in and regulates the absorption of Fe in duodenal mucosal epithelial cells and is regulated by hypoxia or iron content through HIF-2α. Absorbed iron (Fe³⁺) combines with Tf in the circulation, combines with TfRC to enter tissue cells and participates in cell metabolism. hCyb561, human cytochrome b561; Dcytb, duodenal CYB561; ASC, ascorbic acid; HIF-2α, hypoxia-inducible factor-2α; Fe, iron; Tf, transferrin; TfRC, transferrin receptor 1; DMT1, divalent metal transporter 1; CC, colon cancer; LIP, labile iron pool; MDA, monodehydroascorbate.

and may also be a transmembrane reductase of copper (87). The inactivation of the L-gulonolactone oxidase (GULO) gene under physiological conditions prevents the synthesis of the protein GULO enzyme and therefore ASC synthesis. Dcytb can reduce and regenerate oxidized ASC while reducing Fe(3+) to Fe(2+), thereby promoting iron absorption. Therefore, it is speculated that the demand for this reductase increases when the demand for iron absorption is strong (88).

Under normoxic conditions, hemoglobin in the reticulocytes of CYBRD1^{-/-} mice is impaired, and under hypoxic conditions, these mice exhibit reduced duodenal reductase activity and spleen iron reserves, leading to abnormal erythropoiesis (89). As no evidence of hypoxia-induced iron reductase activity has been observed in the CYBRD1^{-/-} mice, this suggests that Dcytb may be the only hypoxia-induced iron reductase in the duodenum. Hypoxia has been demonstrated to strongly upregulate Dcytb expression through a hypoxia-inducible factor-2α (HIF-2α)-dependent mechanism. This activation of HIF-2α also upregulates DMT1 expression, which disrupts local iron homeostasis and promotes cell proliferation, which contributes to the development of colon cancer (90) (Fig. 3). In addition, the upregulation of the iron-import-related proteins Dcytb, DMT1 and TfRC in colorectal cancer cells is associated with increased intracellular iron content (91). An analysis of data from The Cancer Genome Atlas (TCGA) database revealed that the expression

of CYBRD1 is upregulated in ovarian cancer (92), and its increased expression is associated with a reduction in overall survival times (92). In another study it was shown that CYBRD1 is upregulated in glioma cell lines, and its silencing reduces the aggressiveness of glioma cells, indicating its potential as a biomarker for glioma recurrence (93) (Fig. 3). Boulton *et al* (94) reported that the expression of CYBRD1 is upregulated during the progression of Barrett's esophagus to adenocarcinoma, suggesting its involvement in the development of esophageal adenocarcinoma through increased iron absorption. In addition, tumor-initiating cells from the breast and prostate origin have been found to exhibit the altered expression of various genes associated with iron metabolism, including CYBRD1, endothelial PAS domain-containing protein 1/HIF-2α and TfRC (95). By contrast, CYBRD1 has also been reported to exhibit tumor-inhibiting effects. Analysis of gene expression in a Gene Expression Omnibus dataset showed that CYBRD1 expression is downregulated in bladder cancer compared with normal bladder mucosa, suggesting that it may have a tumor-inhibitory role (96). Dcytb has been shown to be an important predictor of prognosis in patients with breast cancer, with high Dcytb expression serving as an overall predictor of distant metastasis-free survival and exhibiting a significant association with increased relapse-free survival (23). However, the role of Dcytb in breast cancer cells was indicated to be unrelated

to iron metabolism; instead it was demonstrated to delay tumor progression by inhibiting the activation of focal adhesion kinase, a kinase that plays a central role in tumor cell adhesion and metastasis (23). In addition, analysis of TCGA data has shown that the CYBRD1 protein level decreases with increasing lung adenocarcinoma stage and grade, indicating a negative correlation between CYBRD1 expression and the progression of lung adenocarcinoma. Moreover, the low expression of CYBRD1 is associated with poor overall and disease-specific survival (97).

LCytb/CYB561A3. This reductase is present in the late endolysosome-lysosome membrane; it is expressed at high levels in the adrenal gland, lymph nodes, B lymphocytes and monocytes, but is downregulated in sepsis (98) and skin warts caused by human papillomavirus infection (99).

LCytb demonstrates iron reductase activity in yeast (67,75). It also participates in iron homeostasis in endosomes and lysosomes. In late endosomes and lysosomes, LCytb uses ASC as an electron donor to reduce Fe^{3+} to Fe^{2+} , which is then transferred by DMT1 into the cytoplasm. In addition, LCytb regulates the iron cycle in macrophages by reducing the expression of lysosomal reductase, which is part of the innate immune response that prevents the proliferation of pathogens and sepsis (100). The knockout of LCytb/CYB561A3 can cause catastrophic damage to lysosomes and mitochondria and damage mitochondrial respiratory function (101). A study evaluated the relationship between LCytb/CYB561A3 and tumors, including Burkitt's lymphoma caused by EBV infection (101). CYB561A3 was demonstrated to be critical for the proliferation of Burkitt's lymphoma cells, but to have no effect on the proliferation of lymphoblastic lymphoma cells or other cancer cells subjected to EBV transformation. Although the autophagic degradation of ferritin and plasma membrane transferrin were upregulated, CYB561A3 knockout induced severe iron starvation in Burkitt's cells (101).

SDR2/FRRS1. SDR2, encoded by the gene FRRS1, is a homolog of CYB561 and Dcytb, which is localized in the cell membrane and expressed at high levels in the liver (62), kidney (62), esophageal mucosa, oral epithelium, gallbladder, testicles and heart, as well as in lesions associated with asthma, dermatitis, and rhinitis (102). A recent study found that FRRS1 is upregulated in cervical squamous cell carcinoma (103). SDR2 exhibits iron chelate reductase activity and functions as an active iron reductase that regulates catecholamines in the brain. It reduces Fe^{3+} to Fe^{2+} prior to transportation from the endosome to the cytoplasm (67,104) (Fig. 3).

FRRS1 has been predicted by machine learning methods to be upregulated in the brain tissue of patients with Alzheimer's disease and suggested to be a potential risk gene for Alzheimer's disease (105). Linton *et al* (106) found that the expression of FRRS1 is downregulated in primary soft tissue sarcoma of the extremities and is negatively correlated with metastatic recurrence. In addition, they tested the combined prognostic effects of FRRS1, helicase 4, complement Factor H and mesenchymal-epithelial transition factor using a simple equal-weight scoring system, and found that they had a greater prognostic effect than tumor grading. However, by contrast,

other researchers found that FRRS1 is upregulated in SiHa and HeLa cervical squamous cell carcinoma cells, and transfection with short hairpin-FRRS1 inhibited the growth of these cells and promoted their apoptosis (103).

101F6/CYB561D2. Encoded by CYB561D2, 101F6 is highly expressed in the pancreas, nervous system, granulocytes and human glioma (107). It is a transmembrane reductase with iron reductase activity (108), which uses ASC in the cytoplasm as an electron donor to transfer electrons through the endoplasmic reticulum for reduction of luminal MDA and Fe^{3+} (109-111) (Fig. 3).

CYB561D2 is a putative tumor suppressor gene that is located in the 3p21.3 region of the human chromosome, where allelic deletions and genomic changes are frequently found in lung cancer and numerous other cancers (112-114). The overlap of heterozygosity deletion and homozygosity deletion in this region occurs frequently in lung and breast cancer, suggesting that one or more genes in this region play an important role in the pathogenesis of these cancers (112-115). Recombinant adenovirus-mediated transfection of 101F6 demonstrated that 101F6 inhibits cell growth alters the cell cycle and induces apoptosis in human lung cancer cells (116). In addition, the exogenous expression of 101F6 enhances the uptake of ASC by lung cancer cells, leading to the accumulation of cytotoxic H_2O_2 . This cooperatively kills tumor cells through apoptosis and autophagy pathways, independently of caspase activation (116). By contrast, the upregulated expression of CYB561D2 has been found to be associated with a higher clinical grade and shorter survival time in patients with glioma. *In vitro* experiments revealed that the overexpression of CYB561D2 in glioma increased the expression of the immunosuppressive genes PD-L1, chemokine (C-C motif) ligand 2 and tryptophan 2,3-dioxygenase in co-cultured T cells (117), while CYB561D2 knockout inhibited the growth, colony formation and migration of glioma cells and promoted cell apoptosis (117).

5. Summary

The Cytb561 family is a class of transmembrane proteins characterized by six transmembrane helices and two heme groups, which exhibit electron transfer and ferric reductase activities. The main members of this family, namely CYB561, Dcytb, LCytb, SDR2 and 101F6, are involved in ASC recycling and iron metabolism. They are widely expressed in human tissues, upregulated or downregulated in different tumors, and involved in the pathogenesis of a variety of diseases and tumors. In addition, they are potential prognostic indicator for certain cancers. Although all members of this family theoretically exhibit iron reductase activity, they are not necessarily involved in iron metabolism in tumors, and Dcytb is a notable example. Members of the Cytb561 family play crucial roles in the promotion or suppression of numerous types of malignant tumors; for example, CYB561 promotes breast cancer growth, Dcytb facilitates glioma invasion and inhibits the growth of breast cancer and bladder cancer, CYB561A3 is crucial in the proliferation of Burkitt's lymphoma cells, and SDR2 accelerates cervical squamous cell carcinoma growth. In addition, 101F6 represses the

growth of human lung cancer cells but contributes to the growth of glioma cells. These transmembrane proteins also have the potential to serve as therapeutic targets in various tumors. The reasons are as follows: i) Their expression levels are up- or downregulated in various tumor tissues compared with corresponding normal tissues, and this differential expression is associated with the prognosis of patients; for example, the upregulated expression of CYB561 in breast cancer is a poor prognostic factor. ii) At the cellular level, altering their expression levels can alter the functional status of tumor cells, such as by promoting or inhibiting cell proliferation, migration, invasion and apoptosis. iii) At the animal level, modulating their expression levels in tumor cells can affect the tumor size in nude mice; for example, the knock-down of CYB561 expression level in HER2-positive breast cancer cells reduced tumorigenicity compared with that in the control (84). The seemingly contradictory roles and tumor specificity of the members of the Cytb561 family suggest that their intricate mechanisms of action in tumors merit further study. At present, the role of this family in tumors and the associated mechanisms are still under investigation. To develop precise targeted treatments for patients with tumors, it is essential to conduct further research to elucidate the role of these iron metabolism-related proteins.

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Authors' contributions

XZ and ZA conceived of the review and acted as mentors and guarantors of the work. XZ wrote the manuscript. HLe was involved in drafting the manuscript. HLi modified the manuscript. XG reviewed and revised the text. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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