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Review

REVIEW

The influence of mitochondrial-directed regulation of Wnt signaling on tumorigenesis

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Abstract

Mitochondria are dynamic organelles that play a key role in integrating cellular signaling. Mitochondrial alterations are evident in all stages of tumorigenesis and targeting mitochondrial pathways has emerged as an anticancer therapeutic strategy. The Wnt-signaling pathway regulates many fundamental cellular functions such as proliferation, survival, migration, stem-cell maintenance, and mitochondrial metabolism and dynamics. Emerging evidence demonstrates that mitochondrial-induced regulation of Wnt signaling provides an additional mechanism to influence cell-fate decisions. Crosstalk between mitochondria and Wnt signaling presents a feedforward loop in which Wnt activation regulates mitochondrial function that, in turn, drives Wnt signaling. In this mini-review, we will discuss the recent evidence revealing the mitochondrial control of Wnt signaling and its implications for tumorigenesis and anticancer therapeutic targeting.

Key words: ββ-catenin; cancer stem cells; metabolic reprogramming; metabolism; PGAM5

Introduction

Mitochondria are dynamic organelles that quickly respond to environmental changes and cellular demands for energy while simultaneously integrating cellular-stress signaling [1]. In addition, mitochondrial metabolism and function serve as key regulators of the differentiation and self-renewal of stem cells, including cancer stem cells [2–6]. Multiple studies provide evidence to support a role for Wnt signaling in the regulation of mitochondrial function. In more recent years, mitochondrial-initiated regulation of Wnt signaling has emerged, suggesting bidirectional crosstalk between mitochondria and the Wnt pathway. Given the crucial role of Wnt signaling in cell-fate decisions including proliferation and survival, mitochondrial-induced regulation of Wnt signaling provides an additional mechanism whereby mitochondria serve as signaling hubs in the cell.

Mitochondria are double-membrane organelles that serve many functions for the cell, but arguably the most important is energy production in the form of adenosine 5′-triphosphate (ATP). In addition, mitochondria play important roles in the induction of apoptosis, calcium regulation, reactive oxygen species (ROS) production, redox balance, production of signal transduction intermediates, and production of epigenetic regulators [1]. All stages of tumorigenesis including initiation, progression, and metastasis exhibit mitochondrial alterations. Altered mitochondrial metabolism is a hallmark of cancer cells and was first described by Warburg as an adaptation to impaired mitochondrial function with enhanced glycolysis despite the presence of oxygen [7]. However, more recent studies convey that cancer metabolism is not the consequence of mitochondrial dysfunction and that cancer cells yield a significant amount of ATP through oxidative phosphorylation. Instead, cancer cells exhibit a hybrid metabolic state, utilizing both oxidative phosphorylation and glycolysis, allowing adaptation to changing microenvironments and the utilization of metabolites and mitochondrial enzymes to create anabolic precursors

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necessary for rapid cell growth [8, 9]. Due to this, targeting mitochondrial pathways has emerged as an anticancer therapeutic strategy to limit crucial ATP production or to induce apoptosis. For instance, the diabetic drug metformin inhibits the electron-transport-chain complex I and reduces overall cancer incidence and mortality rates [10].

Wnt/β-catenin signaling

Canonical and non-canonical Wnt signaling plays a variety of major roles in the cell, such as inducing cell proliferation, metabolic regulation, and directing tissue movements and cell polarization. In the absence of Wnt ligands, β-catenin is phosphorylated at N-terminal serine and threonine sites by a destruction complex consisting of adenomatous polyposis coli (APC), axis inhibition protein 1 (AXIN1), glycogen synthase kinase 3β (GSK3β), and casein kinase 1α (CK1α). Phosphorylated β-catenin is then ubiquitinated and targeted for proteosomal degradation. During canonical Wnt signaling (Figure 1), the binding of Wnt ligands to Frizzled family receptors and the low-density lipoprotein-receptor-related protein 5/6 co-receptors prevents β-catenin phosphorylation and degradation, thereby allowing it to accumulate and translocate into the nucleus. In the nucleus, it activates the T-cell factor (TCF) and lymphoid-enhancer factor (LEF) family of transcription factors, which in turn induce transcription of Wnt target genes known to regulate a variety of cellular functions including proliferation (*MYC*, *CCND1*, *PPARD*), survival (*ASCL2*, *ABCB1*, *BIRC5*), and migration (*MMP7*, *MMP14*). *LGR5* is another Wnt target gene and is important for stem-cell homeostasis in hair follicles, ovarian epithelium, and intestinal epithelium [11]. Another important Wnt target gene is *AXIN2*, which is often used as an indicator of canonical Wnt-pathway activity and negatively regulates Wnt signaling via the degradation of β-catenin [12]. The major Wnt targets in metabolic regulation are pyruvate dehydrogenase kinase 1 (*PDK1*) and monocarboxylate transport protein (*MCT*)-1 [11]. β-catenin physically associates with histone acetylases p300 and cAMP-response element-binding (CREB)-binding protein that integrate and enhance the transcription of multiple signaling pathways via remodeling chromatin into a relaxed state, thereby allowing access by ribonucleic acid (RNA) polymerase II [13].

The β-catenin independent non-canonical pathway is activated when Wnt5α binds to the receptor complex of Frizzled, receptor tyrosine kinase-like orphan receptor (Ror)1/2, or receptor-like tyrosine kinase (Ryk), activating non-canonical signaling such as the Wnt/Ca^{2+} and Wnt/planar pathways. Non-canonical Wnt signaling is involved in the regulation of cell polarity and embryonic development [14, 15]. An important mechanism of Wnt-pathway regulation has been demonstrated by two transmembrane ubiquitin ligases: ring finger protein 43 (RNF43) and zinc and ring finger 3 (ZNRF3). RNF43 and ZNRF3 ubiquitinate Frizzled family receptors, decreasing their cell-surface expression and dampening Wnt signaling [16, 17]. Recent evidence suggests that RNF43 suppresses both canonical and non-canonical Wnt signaling and acts as a tumor suppressor [18]. Due to the influence of Wnt/β-catenin signaling on cell-fate decisions, particularly considering enhanced proliferation, aberrant activation of Wnt signaling promotes carcinogenesis in several organs, predominantly in the colon.

Wnt-pathway genetic mutations in cancer

Multiple cancers exhibit aberrant, constitutively active Wnt/β-catenin signaling driven by genetic mutation or epigenetic modifications of genes in this pathway. The American Association for Cancer Research (AACR) Project GENIE reported that mutation in the *APC* gene is present in 10.3% of all cancer cases, with colorectal cancer (CRC) having the greatest prevalence (present in 49.5% of all CRC patients), followed by non-small-cell lung cancer (NSCLC; present in 5.2% of all NSCLC patients), melanoma (present in 7.9% of all melanoma patients), and breast cancer (present in 2.1% of all breast-cancer patients) [19]. Alterations in the *APC* gene generate truncated mutants lacking all binding sites for AXIN and abolish the formation of the β-catenin destruction complex. In CRC, NSCLC, melanoma, and in ≤70% of certain subtypes of breast cancers, *APC* is hypermethylated, contributing to its inactivation, and is associated with resistance to chemotherapy [19–23].

The majority of CRCs, including sporadic (≤80%) and inflammation-induced (∼50%), carry a genetic mutation in either *APC* or *CTNNB1* (encoding β-catenin) [24–26]. Mutations in *CTNNB1* render β-catenin resistant to phosphorylation that drives it to proteasomal degradation [27]. The AACR Project GENIE reported that mutation in the *CTNNB1* gene is present in 3.2% of all cancer cases, with CRC and uterine corpus neoplasm having the greatest prevalence, followed by NSCLC, melanoma, and hepatocellular cancer [19]. Mutations in *CTNNB1* were more frequent in colorectal tumors lacking mutation in the *APC* gene, whereas mutation of both *CTNNB1* and *APC* in the same tumor was rare, suggesting that mutation of only one was sufficient to confer Wnt-pathway activation [28].

Genetic mutation in *AXIN1* is less frequent than *APC* or *CTNNB1* mutations in multiple cancers including CRC, NSCLC, breast cancer, melanoma, and hepatocellular cancer [19, 29]. Similarly, mutation in *GSK3β* is even less frequent than *AXIN1* mutation in CRC, NSCLC, melanoma, malignant glioma, and breast cancer [19]. Mutation in the *RNF43* gene is present in 3.2% of all cancer cases, with CRC and breast cancer having the greatest prevalence, followed by uterine corpus neoplasm, pancreatic cancer, and NSCLC [19]. Mutations in the *RNF43* gene were demonstrated to play a key role in the formation of tumors in subtypes of CRC, pancreatic ductal adenocarcinoma, and endometrial cancer [13]. Truncation mutations in CREB-binding protein have been identified in ovarian tumors, NSCLC, lymphoma, leukemia, bladder cancer, and CRC cell lines [30–35]. In addition, *EP300* (encodes p300) mutation or loss of heterozygosity have been demonstrated in many types of cancer and influences sensitivity to chemotherapy and stemness [36, 37].

Wnt regulation of mitochondrial function

It is well established that Wnt signaling modulates mitochondrial function, including biogenesis, metabolism, and dynamics in non-transformed and cancer cells. For instance, increased Wnt/β-catenin signaling activates mitochondrial biogenesis and increases mitochondrial-derived ROS production and oxidative damage in mouse embryonic fibroblasts and non-transformed C2C12 cells [38]. Similar results were shown with decreased mitochondrial biogenesis during β-catenin knock-down in breast-cancer cells [39]. Further, a report showed that Wnt3α is involved in the regulation of mitochondrial biogenesis in adipocytes [40]. Mice administered Wnt3α exhibited higher expression of genes associated with mitochondria regulation and increased numbers of mitochondria, which was shown to be dependent on p38-MAPK (mitogen-activated protein kinases) and CREB signaling [40]. A study suggested that phosphoglycerate mutase 5 (PGAM5), a mitochondrial phosphatase, is involved in the biogenesis of mitochondria through activation of the Wnt/β-

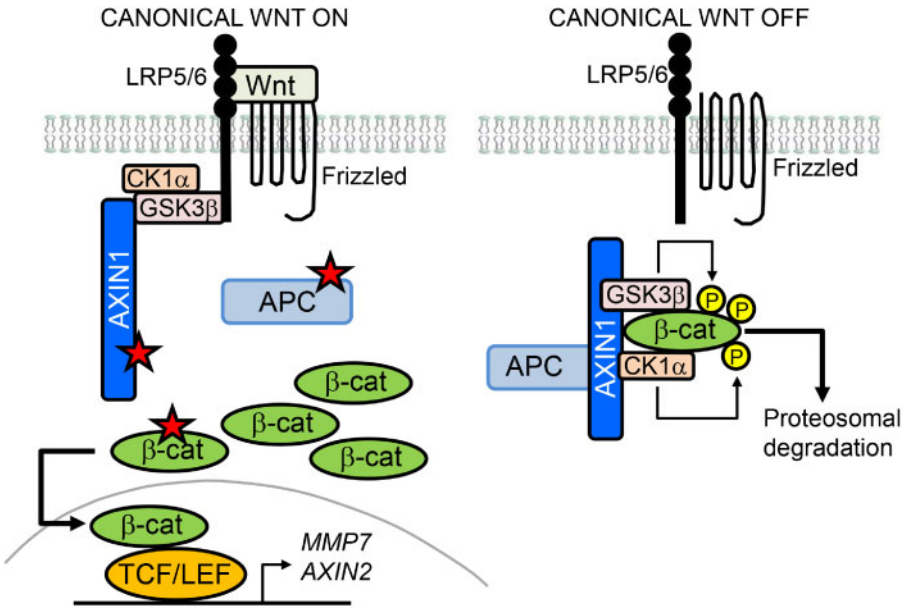

Figure 1. Canonical Wnt signaling. In the presence of Wnt, or in cells harboring genetic mutations in *APC*, *CNNTB1* (encoding β-catenin) or *AXIN1* genes (indicated by red star), β-cat accumulates in the cytosol, translocates to the nucleus, and activates the Wnt-transcriptional program. In the absence of Wnt ligand, β-cat is phosphorylated by GSK3β and CK1α and targeted by the degradation complex formed via interaction with APC and AXIN for proteosomal degradation. APC, adenomatous polyposis coli; β-cat, β-catenin; CK1α, casein 1 alpha; CNNTB1, catenin beta 1; GSK3β, glycogen synthase kinase 3 beta; LEF, lymphoid enhancer factor; LRP5/6, low-density lipoprotein receptor related protein 5/6; MMP7, matrix metalloproteinase 7; TCF, T-cell factor; Wnt, wingless/integrated.

catenin pathway by replenishing mitochondria as a result of mitochondrial stress [41]. The colocalization of PGAM5 remains controversial, but evidence suggests that it can be colocalized to the inner mitochondrial membrane, outer mitochondrial membrane, or both [41]. If the mitochondria experiences loss of its membrane potential, PGAM5 is cleaved by presenilin-associated rhomboid-like protein (PARL) and released into the cytosol. PGAM5 then binds to AXIN, which results in the dephosphorylation and stabilization of β-catenin. Increased levels of cytosolic PGAM5 also led to increased transcriptional activity of the Wnt/β-catenin pathway [41]. This process degrades damaged mitochondria and, in turn, replenishes the number of mitochondria, leading to an increase in the quantity and quality of mitochondria [41]. These results imply that activation of Wnt/β-catenin signaling by PGAM5 is independent of upstream stimulation by Wnt ligands and is activated as a result of mitochondrial damage. Thus, Wnt/β-catenin-pathway activation by PGAM5 plays two key roles in mitochondrial homeostasis by inducing mitochondrial biogenesis and mitophagy.

Wnt signaling has also been implicated in regulating mitochondrial metabolism and permeabilization. Inhibition of Wnt3α was shown to decrease the mitochondrial metabolism in adipocytes [42]. Oncogenic Wnt signaling in CRC cells also modulates mitochondrial metabolism via glycolysis for the production of anabolic precursors necessary for rapid cancer-cell growth [43]. AXIN2, a Wnt target gene, localizes to the mitochondrial electron-transport-chain complex IV and decreases its activity, ATP production, and cell proliferation in HeLa cells [44]. The disruption of Wnt signaling has been linked to several neurodegenerative diseases, which are commonly associated with mitochondrial dysfunction, such as Alzheimer's disease [45, 46]. Alzheimer's disease is characterized by the presence of extracellular depositions of Aβ oligomer aggregates that induce permeabilization of mitochondrial membranes via the mitochondrial permeability transition pore (mPTP) [46]. It was recently demonstrated that canonical Wnt signaling prevents Aβ oligomer-induced mPTP opening, protecting hippocampal neurons from death, suggesting that Wnt activation may act as a therapeutic target in Alzheimer's-disease patients by directly influencing the mitochondria [45].

Wnt signaling has been demonstrated to regulate mitochondrial distribution within the cell and mitochondrial dynamics such as fission and fusion. Mitochondrial homeostasis is maintained when fission and fusion are in balance, ATP production is optimal, and the integrity of the mitochondrial genome is preserved [47]. Disruption of these processes can lead to mitochondrial dysfunction. Both canonical and non-canonical Wnt signaling has been shown to regulate mitochondrial distribution and dynamics [48]. In stem cells, Wnt signaling has been reported to play a role in regulating mitochondrial dynamics, pluripotency, and apoptosis [49]. In a mouse model of *Cisd2* deletion characterized by dysfunctional electron-transport-chain activity, induced pluripotent stem cells exhibited increased Ca^{2+} levels, which in turn negatively regulated the Wnt/β-catenin pathway, generated mitochondrial ultrastructural abnormalities such as underdeveloped cristae, and decreased the overall numbers of mitochondria [49]. Knock-down of the Wnt target gene *CCND1* (encoding Cyclin D1) in human SW480 CRC cells caused mitochondria to distribute homogeneously in the cytosol, as opposed to control cells that portrayed a normal perinuclear mitochondrial distribution [50]. In addition, CCDN1 knock-down also altered the mitochondrial mass and elevated levels of ATP, implying that Cyclin D1 has an effect on mitochondrial metabolism [50]. Mitochondrial distribution was also shown to be regulated by APC, which localized to mitochondria and initiated mitochondrial transport to locations within the cell in need of increased energy production and was shown to be crucial for cell migration [51]. Mitochondrial responsiveness to Wnt in melanoma cells was demonstrated to be dependent on the mutation status of phosphatase and tensin homolog (PTEN), a lipid and protein phosphatase [52]. PTEN wild-type melanoma cells displayed a normal perinuclear mitochondrial localization that was disrupted by knock-down of β-catenin,

suggesting the key involvement of β-catenin in the mitochondrial distribution in these cells. Wnt3α treatment in PTEN wild-type melanoma cells induced larger and more elongated mitochondria, elevated expression of mitochondrial fusion proteins, and altered mitochondrial-membrane potential that was not evident in PTEN mutant cells, suggesting the Wnt regulation of mitochondrial dynamics, structure, and morphology is dependent on PTEN [52].

Mitochondrial regulation of the Wnt pathway in cell homeostasis and tumorigenesis

In addition to Wnt action on mitochondria, recent evidence suggests that mitochondrial retrograde signaling directly regulates the Wnt pathway, revealing bidirectional crosstalk between mitochondria and Wnt signaling. These studies reveal an important node of mitochondrial-induced signaling that can influence cell-fate decisions crucial for cell homeostasis and the progression of tumorigenesis (Figure 2).

Cell homeostasis

Secreted Wnt was recently been demonstrated to function as a 'mitokine' signal, inducing the mitochondrial unfolded protein response (mtUPR) in a cell non-autonomous manner from the nervous system to the periphery [56]. This facilitates the coordination of stress responses across different systems in the body. Using *Caenorhabditis elegans* as a model, Zhang *et al.* showed that mtUPR in neurons induces the secretion of the Wnt ligand EGL-20 (Wnt16b in humans) dependent on the retromer complex component MIG-14 [56]. Neuronal secreted EGL-20 binds to Frizzled receptors on recipient cells and elicits canonical Wnt signaling, resulting in β-catenin activation that was sufficient to induce mtUPR in recipient cells [56]. This study reveals a mechanism whereby Wnt facilitates cell communication relaying mitochondrial stress signals, enabling a whole-organism response to defend against local mitochondrial dysfunction.

Mitochondrial signaling is emerging as an important regulator of the Wnt pathway that in turn is crucial for the maintenance of stem/progenitor cells. Intestinal stem cells are responsible for the high rate of regeneration of intestinal epithelial cells [57]. They are essential in ensuring the homeostasis of the epithelial environment whereby the microbiome and the immune system are constantly interacting with the epithelial cells. Properly functioning mitochondria are key to maintaining homeostasis in intestinal stem cells. One of the main causes of mitochondrial dysfunction is the presence of unfolded proteins that trigger the unfolded protein response, which in turn activates the transcription factor C/EBP homologous protein [47, 58]. As a result, mitochondrial function is compromised due to an increase in oxidative stress. Heat-shock protein 60 (Hsp60) is a mitochondrial chaperone that is important in folding proteins and reducing the level of oxidative stress [2]. Berger *et al.* [2] demonstrated that mitochondrial function, the structure of mitochondrial cristae, and the stemness of epithelial cells in the

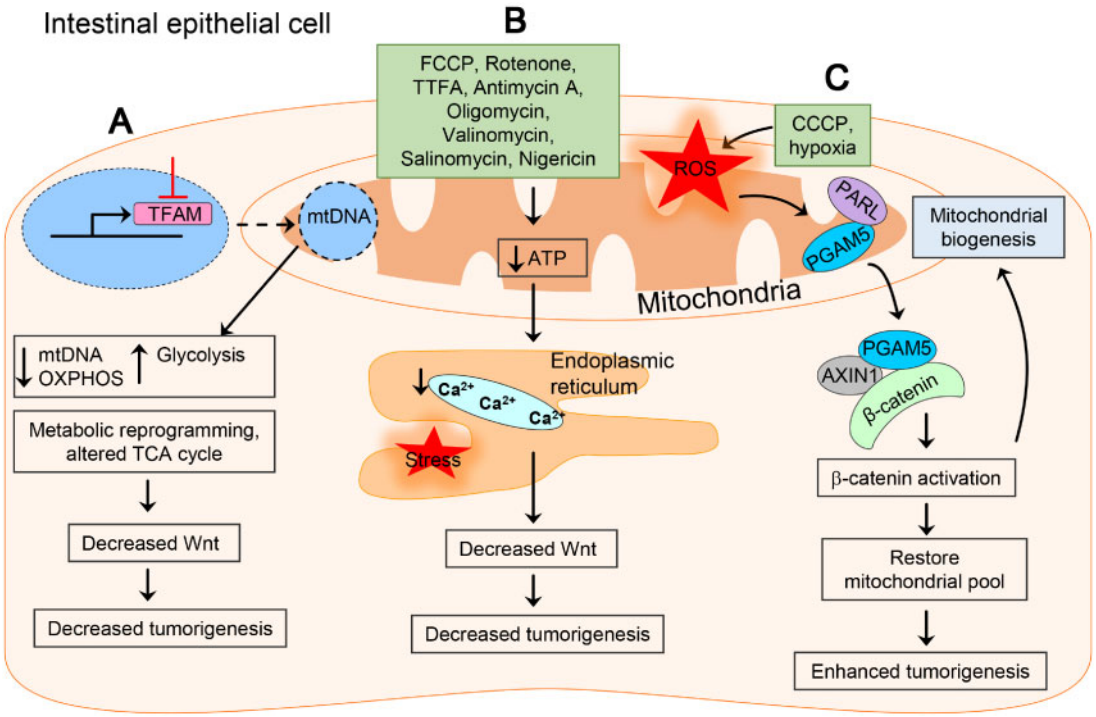

Figure 2. Mitochondrial regulation of β-catenin/Wnt signaling in intestinal epithelial cells influences tumorigenesis. (A) TFAM deficiency induces loss of mtDNA, increases glycolysis, and decreases OXPHOS. Increased expression of the TCA-cycle metabolites α-ketoglutarate suppresses Wnt signaling and tumorigenesis [53]. (B) Multiple drugs used to cause mitochondrial stress and decrease ATP production, such as FCCP, rotenone (inhibits complex I of the electron-transport chain), TTFA (inhibits complex II), antimycin A (inhibits complex III), oligomycin (inhibits complex IV), valinomycin (K^+ ionophore), salinomycin, and nigericin (K^+/H^+) exchangers, induce endoplasmic reticulum stress, and suppress Wnt and tumorigenesis [54]. (C) PGAM5 is released from the mitochondria during stress induced by CCCP or hypoxia and translocates to the cytosol, where it binds to AXIN1, an inhibitor of β-catenin. This binding of PGAM5 to AXIN1 activates β-catenin to upregulate mitochondrial biogenesis to restore the damaged mitochondrial population. However, increased mitochondrial biogenesis could drive CRC tumorigenesis as a means to meet the energy production of cancer cells [55]. ATP, adenosine triphosphate; CCCP; carbonyl cyanide m-chlorophenyl hydrazine; CRC, colorectal cancer; FCCP, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone; mtDNA, mitochondrial DNA; OXPHOS, oxidative phosphorylation; PARL, presenilin-associated rhomboid-like protein; PGAM5, phosphoglycerate mutase 5; ROS, reactive oxygen species; TCA, tricarboxylic acid; TFAM, transcription factor A; TTFA, thenoyltrifluoroacetone.

crypt are altered in mice with intestinal epithelial cell-specific deletion of *Hsp60*. Interestingly, this mechanism does not involve C/EBP homologous protein. However, the decrease in stemness or proliferation and increase in mitochondrial dysfunction can be mitigated or compensated for by the paracrine release of Wnt-signaling molecules involving crypts spared from *Hsp60* gene deletion. This Wnt signaling from Hsp60 wild-type crypts induces hyperproliferation to sustain a healthy epithelial cell population [2]. Although aberrant Wnt signaling is implicated in the proliferation of cancer cells [13], the increased release of signaling molecules related to Wnt, such as Wnt10a and Rspo1, was proven to be beneficial in rescuing intestinal epithelial mitochondria. Wnt10a in particular increased the expression level of Lgr5, a marker of intestinal stem cells, in the intestinal crypt in the absence of *Hsp60*. Taken together, Hsp60 deficiency drives compensatory hyperproliferation by neighboring wild-type crypts via Wnt-associated signaling molecules [2].

Mitochondrial control of Wnt regulates the differentiation of neural progenitor cells. Low levels of mitochondrial-derived ROS were shown to play an important role in Wnt signaling and the cell fate of human neural progenitor ReNcell VM197 cells (hNPCs) [59]. At low levels, ROS have been known to act as a second messenger for a variety of processes and to drive redox-dependent procedures [60]. Specifically in hNPCs, mitochondrial-derived ROS interacts with the effector Dishevelled and Nucleoredoxin complex, causing Dishevelled to dissociate from Nucleoredoxin and in turn stimulate the Wnt/β-catenin pathway [59]. By monitoring the intracellular redox balance state, the production of ROS in the mitochondria was shown to be increased during the differentiation phase of hNPCs. Mitochondrial-derived ROS induction of the Wnt/β-catenin pathway via Dishevelled-2 dissociation from Nucleoredoxin, and in turn differentiation of hNPCs, was shown to be dependent on the mitochondrial uptake of Ca^{2+} released from the endoplasmic reticulum [59]. This study reveals a new mechanism involving mitochondrial-derived ROS regulation of the Wnt-signaling pathway in modulating neuronal-cell differentiation vs proliferation.

Progression of tumorigenesis

A recent study by Bernkopf *et al.* [41] using HEK293T, HeLa, U2OS, SW480, and C2C12 cells discovered a cell-intrinsic signaling pathway originating from mitochondria that activates the Wnt/β-catenin pathway independently of Wnt ligands. During mitochondrial stress (loss of mitochondrial-membrane potential) induced by the electron-transport-chain uncoupler carbonyl cyanide m-chlorophenyl hydrazine (CCCP) or exposure to hypoxia, PGAM5, a serine/threonine phosphatase that localizes to the inner mitochondrial membrane, was cleaved by the mitochondrial protease PARL. Cleavage of PGAM5 releases it from the mitochondrial membrane and allows translocation to the cytosol [61]. In the cytosol, PGAM5 interacted with AXIN1 and activated Wnt/β-catenin signaling by promoting the dephosphorylation of β-catenin [41]. As mentioned above, activated β-catenin via PGAM signaling was shown to stimulate mitochondrial biogenesis, as measured by increased mitochondrial numbers, proposed to replenish the damaged mitochondrial pool during stress [41]. In this way, PGAM5 acts to restore mitochondrial homeostasis. Indeed, earlier studies suggested that PGAM5 also modulates mitophagy, which is the process of recycling/removing damaged mitochondria through the autophagy pathway [62, 63]. PGAM5 was able to increase transcriptional activation by wild-type β-catenin but not β-catenin harboring mutation at the N-terminal phosphorylation sites. In PGAM5- or PARL-deficient cells, β-catenin dephosphorylation was diminished during mitochondrial stress, revealing that PGAM5 and PARL are necessary for this mitochondrial-induced regulation of β-catenin [41]. Cells with healthy mitochondria exhibited low levels of PGAM5 and β-catenin phosphorylation by CK1α and GSK3β scaffolded by AXIN1. This revealed that AXIN1 can promote or inhibit β-catenin phosphorylation and degradation, depending on its interaction with CK1α and GSK3β (default state; promotion of β-catenin degradation) or PGAM5 (during mitochondrial stress; inhibit β-catenin degradation). This study demonstrated an important feedback loop involving PGAM5 activation originating in the mitochondria and stimulated during mitochondrial stress that activates β-catenin independently of Wnt ligands to promote restoration of the mitochondrial pool. The authors speculate that this mitochondrial-induced mitochondrial biogenesis could be associated with CRC tumorigenesis as a means to meet the energy-production needs of rapidly growing cancer cells and to perpetuate the rate of DNA damage and tumor progression via increased ROS production due to increased mitochondrial numbers [55].

The intestine is a recognized model for studying the role of Wnt in tumorigenesis, since Wnt signaling is essential for the maintenance of the intestinal epithelium. Mitochondrial retrograde signaling was recently demonstrated to regulate Wnt signaling in CRC [53]. Silencing of transcription factor A, mitochondrial (TFAM), which regulates mitochondrial DNA (mtDNA) replication and transcription, in DLD1 or HCT116 CRC cells with aberrant activation of Wnt signaling causes loss of mtDNA, deficiency of oxidative phosphorylation, and enhanced glycolysis [53]. This was associated with decreased expression of Wnt/β-catenin target genes expressed in CRC-cancer stem cells such as *LGR5*, *CD44*, *TCF7*, *MYC*, and *CD133*. During TFAM silencing, the number of tumor spheroids able to form from HCT116 cells was decreased, as was the number of *APC/Kras* mutant organoids, suggesting that oxidative phosphorylation is necessary for the maintenance of CRC stem cells. Interestingly, TFAM silencing was shown to cause metabolic reprogramming and an altered level of tricarboxylic acid (TCA)-cycle metabolites with increased production of α-ketoglutarate. α-ketoglutarate, in turn, suppressed Wnt signaling via a mechanism dependent on decreased Hif1α expression [53]. To test the effect of the loss of mitochondrial respiration on tumorigenesis *in vivo*, xenograft growth of TFAM knock-down HCT116 cells was measured in severe combined immunodeficiency (Scid) mice. Both the initiation and the growth of xenograft tumors were inhibited in TFAM knock-down cells. Furthermore, mice with intestinal epithelial-specific deletion of *Tfam* exhibited fewer tumors and decreased Wnt/β-catenin target-gene expression in the *Apc*-driven mouse model of intestinal tumorigenesis, suggesting that mitochondrial respiration is crucial for CRC associated with aberrant Wnt activation [53]. Heterozygous deletion of *Tfam* in intestinal epithelial cells did not alter tumorigenesis in the *Apc*-driven mouse model [53]. However, an earlier study using global heterozygous *Tfam* mice crossed with $Apc^{min/+}$ mice demonstrated increased mtDNA instability, mitochondrial-derived ROS production, and small-intestinal, but not colonic, tumor number and growth without enhanced Wnt/β-catenin signaling [64]. These opposing results of *Tfam* heterozygous deletion could be due to whole-body deletion contributing to increased ROS leading to enhanced tumorigenesis vs specific deletion in the intestinal epithelium. Analysis of the Cancer Genome Atlas RNA sequencing data set revealed that TFAM is significantly increased in CRC and associated with Wnt signaling in CRC

patients [53]. These results suggested that TFAM-dependent mitochondrial respiration plays a key role in regulating Wnt signaling in CRC.

Additional mitochondrial regulation of Wnt signaling was demonstrated to be mediated by mitochondrial ATP production [54]. Using sublethal doses of various drugs that alter mitochondrial function (mitochondrial uncouplers, inhibitors of respiratory-chain complexes, inhibitor of K^+ fluxes to affect the mitochondrial-membrane potential), Costa *et al.* showed that these drugs decreased mitochondrial ATP production and decreased Wnt reporter activity in zebrafish and HEK293 cells and the CRC cell line HCT116 cells [54]. This reduced mitochondrial ATP production, decreased Ca^{2+} stores in the endoplasmic reticulum via altered sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity, and induced endoplasmic reticulum stress. Restoration of mitochondrial ATP synthesis or inhibition of endoplasmic reticulum stress rescued Wnt activity in cells treated with the various drugs that alter mitochondrial function [54]. These results reveal a link between mitochondrial ATP metabolism and Wnt-pathway regulation. The authors speculate that inhibition of mitochondrial function, thereby decreasing mitochondrial ATP production, could be beneficial in Wnt-dependent cancers.

Aging, Wnt, mitochondrial function, and tumorigenesis

It is well established that mitochondrial quality and function decline with normal aging and in turn regulate longevity [65]. Risk of cancer development is increased with normal aging and it has been proposed that the decline in mitochondrial function, specifically oxidative phosphorylation, is an important event in cancer initiation [66]. Additionally, with normal aging, in the intestine, Wnt expression and signaling are decreased in intestinal stem cells themselves, surrounding mesenchymal cells, and crypt Paneth cells, resulting in a decline in intestinal stem-cell-regenerative capacity [67]. Therefore, mitochondrial function and Wnt signaling are both decreased upon aging and these observations suggest that mitochondria influence key alterations of aging including Wnt signaling. How, then, is CRC tumorigenesis increased with aging given the frequent Wnt dependence in CRC? This is because Wnt-driven CRC is derived from mutations in Wnt-pathway genes accumulated during normal aging, causing aberrant Wnt activation [68]. Emerging evidence suggests an early loss of mitochondrial function during preneoplasia and restoration after malignant transformation [69–71], suggesting that mitochondrial-driven Wnt likely returns in fully transformed cells regardless of aging but this has yet to be demonstrated. Additionally, decreasing mitochondrial function to target established Wnt-signaling-dependent CRC might be a therapeutic approach.

Therapeutic targeting of mitochondrial/Wnt Signaling

Given the complexity of cancer in which the targeting of more than one molecular pathway can result in a more effective therapeutic response, targeting mitochondrial signaling in combination therapy with currently used chemotherapeutics may sensitize chemoresistant cells. Indeed, emerging evidence suggests that targeting mitochondrial pathways sensitizes many types of tumor cells resistant to standard treatment [72–79]. Our understanding of the role of mitochondrial signaling in regulating Wnt-pathway activation is incomplete. However, recent studies have suggested important implications for mitochondria-directed Wnt-signaling regulation in tumorigenesis. Future studies are needed to determine whether targeting mitochondrial function could provide an effective therapy against Wnt-dependent cancers. Promising results were demonstrated using a mitochondrial uncoupler in mutant β-catenin HCT116 CRC cells resulting in apoptosis and xenograft tumor regression, but not in A375 cells with wild-type β-catenin [80]. Pyrvinium pamoate, a drug approved by the US Food and Drug Administration to treat pinworms that inhibits the electron-transport chain and ATP production [81], was shown to block Wnt/β-catenin signaling *in vivo* in Xenopus and *in vitro* in Wnt-dependent CRC cell lines HCT116 and SW480 by specifically targeting CK1α [82]. Interestingly, SW480 cells with restoration of full-length APC and normal Wnt signaling were 80-fold less sensitive to cell death induced by pyrvinium pamoate compared with SW480 cells with truncated APC and aberrant Wnt activation [82]. Additionally, mitochondrial-targeted metal complexes show potential as anticancer therapy via stimulating the loss of mitochondrial-membrane potential and increasing mitochondrial-derived ROS production [83]. A recent study demonstrated that a mitochondrial-targeted platinum complex inhibited cancer-cell proliferation and migration dependent on the blockade of β-catenin activation [84]. Metformin, which inhibits the electron-transport-chain complex I, was shown to inhibit the Wnt/β-catenin pathway and growth of HCT116 and HT29 CRC cells with aberrant Wnt activation [85]. Collectively, these results suggest that targeting mitochondrial pathways is especially deleterious in Wnt-dependent cancers, perhaps via blocking the feedforward-signaling loop generated between mitochondria and Wnt.

Conclusions

Many inhibitors of the Wnt/β-catenin pathway already exist, with some reaching early clinical trials [86]. However, many adult healthy tissues rely on Wnt for renewal and homeostasis. For this reason, Wnt-targeting compounds exhibit adverse reactions, with the intestine seeming to be the most vulnerable, impeding the advancement of these compounds to the late clinical-trial stage [87]. In this regard, targeting mitochondrial pathways altered in cancer cells [7] may provide a novel mechanism to inhibit Wnt-dependent cancers that may avoid the toxicity of current Wnt-targeting compounds. Mitochondrial/Wnt crosstalk provides an exciting therapeutic anticancer target and future studies will reveal the clinical potential of targeting the mitochondrial-directed regulation of Wnt in tumorigenesis.

Authors' contributions

Concept and design: ALT. Drafting the manuscript: YDD, KMA, ALT.

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Conflicts of interest

None declared.

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