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

Yaritza Delgado-Deida, Kibrom M. Alula and Arianne L. Theiss

Division of Gastroenterology and Hepatology, Department of Medicine, University of Colorado School of Medicine, Aurora, CO 80045, USA

*Corresponding author. Division of Gastroenterology and Hepatology, University of Colorado School of Medicine, 12700 East 19th Avenue, RC2 Campus Box: B158 HSC, Aurora, CO 80045, USA. Tel: +1-303-724-7254; Email: arianne.theiss@cuanschutz.edu

Abstract

Mitochondria are dynamic organelles that play a key role in integrating cellular signaling. Mitochondrial alterations are evident at 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, which, 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. 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...
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), glycosynthetic kinase 3β (GSK3β), and casein kinase 1s (CK1s). 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, ABCG1, 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 Wnt5a 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/Ca2+ 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 (ZNF3). RNF43 and ZNF3 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 hyper-methylated, 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 proteosomal 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 Wnt3a is involved in the regulation of mitochondrial biogenesis in adipocytes [40]. Mice administered Wnt3a exhibited higher expression of genes associated with mitochondrial 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 (PGAMS), a mitochondrial phosphatase, is involved in the biogenesis of mitochondria through activation of the Wnt/β-
Mitochondria and Wnt-signaling crosstalk

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-catenin accumulates in the cytosol, translocates to the nucleus, and activates the Wnt-transcriptional program. In the absence of Wnt ligand, β-catenin 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-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 PGAMS 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, PGAMS is cleaved by presenilin-associated rhomboid-like protein (PARL) and released into the cytosol. PGAMS then binds to AXIN, which results in the dephosphorylation and stabilization of β-catenin. Increased levels of cytosolic PGAMS 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 PGAMS is independent of upstream stimulation by Wnt ligands and is activated as a result of mitochondrial damage. Thus, Wnt/β-catenin-pathway activation by PGAMS 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 Ca2+ 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, CCND1 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, valinomycin, 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 hyperpolarization 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 hyperpolarization 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 Cr³⁺ 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 increased 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⁺/− 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 Ca2+ stores in the endoplasmic reticulum via altered sarcoplasmic/endoplasmic reticulum Ca2+-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-independent 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.

Funding

This work is supported by National Institutes of Health grant R01-DK117001 (to A.L.T.) and Litwin IBD Pioneers Crohn’s Colitis Foundation 391869 (to A.L.T.).

Conflicts of interest

None declared.
References

1. Jackson DN, Theiss AL. Gut bacteria signaling to mitochon-
     dria in intestinal inflammation and cancer. Gut Microbes 2019;
     10, doi: 10.1080/19480967.2019.1592421.
2. Berger E, Bath E, Yuan D et al. Mitochondrial function controls
     intestinal epithelial stemness and proliferation. Nat Commun
     2016;7:13171.
3. Ito K, Hiroa A, Arai F et al. Reactive oxygen species act through
     p38 MAPK to limit the lifespan of hematopoietic stem cells. Nut
     Med 2006;12:446–51.
4. Khacho M, Clark A, Svoboda DS et al. Mitochondrial dynamics
     impacts stem cell identity and fate decisions by regulating a
     nuclear transcriptional program. Cell Stem Cell 2016;19:222–47.
5. Morshedi CM, Reynolds BA, Craig CG et al. Neural stem cells in
     the adult mammalian forebrain: a relatively quiescent sub-
     population of subependymal cells. Neuron 1994;13:1071–82.
6. Peizoto J, Lima J. Metabolic traits of cancer stem cells. Dis
     Model Mech 2018;11.
7. Liberti MV, Locasale JW. The Warburg effect: how does it ben-
     efit cancer cells? Trends Biochem Sci 2016;41:211–8.
8. Ward PS, Thompson CB. Metabolic reprogramming: a cancer
     hallmark even Warburg did not anticipate. Cancer Discov
     2016;6:195–200.
9. Tsukiyama T, Fukui A, Terai S et al. Reactive oxygen species
     mediates response to chemotherapeutic agents in breast cancer.
     BMC Cancer 2015;15:457.
10. Seo YS, Kim YJ, Kim MS et al. Association of mitofusin use
     with cancer-specific mortality in hepatocellular carcinoma
     after curative resection: a nationwide population-based
     study. Medicine (Baltimore) 2016;95:e5327.
11. Ramakrishnan AB, Cadigan KM. Wnt target genes and where
     to find them. F1000Res 2017;6:746.
12. Jho EH, Zhang T, Demott C et al. Wnt/beta-catenin/Tcf signaling
     induces the transcription of Axin2, a negative regulator of the
     signaling pathway. Mol Cell Biol 2002;22:1172–83.
13. Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. Oncogene
     2017;36:1461–73.
14. Okamoto M, Udagawa N, Uehara S et al. Noncanonical Wnts
     enhances Wnt/beta-catenin signaling during osteoblastogen-
     esis. Sci Rep 2015;4:4493.
15. Wang Y. Wnt/Planar cell polarity signaling: a new paradigm for
     cancer therapy. Mol Cancer Ther 2009;8:2103–9.
16. Hao HX, Xie Y, Zhang Y et al. ZNRF3 promotes Wnt receptor
     turnover in an R-spondin-sensitive manner. Nature 2012;485:
     195–200.
17. Koo BK, Spit M, Jordens I et al. Tumour suppressor RNF43 is a
     stem-cell E3 ligase that induces endocytosis of Wnt recep-
     tors. Nature 2012;488:665–9.
18. Tsukiyama T, Fukui A, Terai S et al. Molecular role of RNF43 in
     canonical and noncanonical Wnt signaling. Mol Cell Biol 2015;
     35:2007–23.
19. Consortium A. AACR Project GENIE: powering precision medicine
     through an international consortium. Cancer Discov 2017;7:818–31.
20. Guo S, Tan L, Pu W et al. Quantitative assessment of the diag-
     nostic role of APC promotor methylation in non-small cell lung
     cancer. Clin Epigenetics 2014;6:5.
21. Liang TJ, Wang HX, Zheng YY et al. APC hypermethylation for
     early diagnosis of colorectal cancer: a meta-analysis and lit-
     erature review. Oncotarget 2017;8:46468–79.
22. VanKloppenberg MK, Bedalov CO, Soto KF et al. APC selec-
     tively mediates response to chemotherapeutic agents in
     breast cancer. BMC Cancer 2015;15:457.
23. Worm J, Christensen C, Grønbæk K et al. Genetic and epige-
     netic alterations of the APC gene in malignant melanoma.
     Oncogene 2004;23:5215–26.
24. Conlin A, Smith G, Carey FA et al. The prognostic significance
     of K-ras, p53, and APC mutations in colorectal carcinoma. Gut
     2005;54:1283–6.
25. Fearon ER, Vogelstein B. A genetic model for colorectal tu-
     morigenesis. Cell 1990;61:759–67.
26. Yamauchi M, Morikawa T, Kuchiba A et al. Assessment of co-
     lorectal cancer molecular features along bowel subsites chal-
     lenges the conception of distinct dichotomy of proximal
     versus distal colorectum. Gut 2012;61:847–54.
27. Tortelote GG, Reis RR, de Almeida Mendes F et al. Complexity
     of the Wnt/beta-catenin pathway: searching for an activation
     model. Cell Signal 2017;40:30–43.
28. Sparks AB, Morin PJ, Vogelstein B et al. Mutational analysis of the
     APC/beta-catenin/Tcf pathway in colorectal cancer. Cancer Res
     1998;58:1130–4.
29. Mazzoni SM, Fearon ER. AXIN1 and AXIN2 variants in gastro-
     intestinal cancers. Cancer Lett 2014;355:1–8.
30. Gyu I, Guo G, Huang Y et al. Frequent mutations of chromatin
     remodeling genes in transitional cell carcinoma of the blad-
     der. Nat Genet 2011;43:875–8.
31. Kishimoto M, Kohno T, Okudela K et al. Mutations and dele-
     tions of the CBG gene in human lung cancer. Clin Cancer Res
     2005;11:512–9.
32. Morin RD, Mendez-Lago M, Mungall AJ et al. Frequent muta-
     tion of histone-modifying genes in non-Hodgkin lymphoma.
     Nature 2011;476:299–303.
33. Mullighan CG, Zhang J, Kasper LH et al. CREBBP mutations in
     relapsed acute lymphoblastic leukaemia. Nature 2011;471:
     235–9.
34. Ward R, Johnson M, Shridhar V et al. CBP truncating muta-
     tions in ovarian cancer. J Med Genet 2005;42:514–8.
35. Bordonaro M, Lazarova DL. CREB-binding protein, p300, buty-
     rate, and Wnt signaling in colorectal cancer. World J Gastroenterol
     2015;21:8238–48.
36. Gayther SA, Batley SJ, Linger L et al. Mutations truncating the
     EP300 acetylase in human cancers. Nat Genet 2000;24:300–3.
37. Asaduzzaman M, Constantinos S, Min H et al. Tumour sup-
     pressor EP300, a modulator of paclitaxel resistance and stem-
     ness, is downregulated in metastatic breast cancer. Breast
     Cancer Res Treat 2017;163:461–74.
38. Yoon JC, Ng A, Kim BH et al. Wnt signaling regulates mito-
     chondrial physiology and insulin sensitivity. Genes Dev 2010;
     24:1507–18.
39. Vergara D, Stanca E, Guerra F et al. Beta-catenin knockdown
     affects mitochondrial biogenesis and lipid metabolism in
     breast cancer cells. Front Physiol 2017;8:544.
40. Ning X, He J, Shi X et al. Wnt3a regulates mitochondrial bio-
     genesis through p38/CREB pathway. Biochem Biophys Res
     Commun 2019;516:1019–25.
41. Bernkopf DB, Jalal K, Bruckner M et al. Secreted frizzled-related
     protein 5 suppresses adipocyte mitochondrial metabolism
     through WNT inhibition. J Clin Invest 2012;122:2405–16.
42. Mori H, Prestwich TC, Reid MA et al. Secreted frizzled-related
     protein 5 suppresses adipocyte mitochondrial metabolism
     through WNT inhibitors. J Clin Invest 2016;122:2405–16.
43. Pate KT, Stringari C, Sproll-Tanio S et al. Wnt signaling
     directs a metabolic program of glycolysis and angiogenesis in
     colon cancer. EMBO J 2014;33:1454–73.
44. Shin JH, Kim HW, Rhyu IJ et al. Axin is expressed in mitochon-
     dria and suppresses mitochondrial ATP synthesis in HeLa
     cells. Exp Cell Res 2016;340:12–21.
45. Arrazola MS, Ramos-Fernandez E, Cisternas P et al. Wnt signal permeability ablates oligomer-induced mitochondrial permeability transition pore opening preserving mitochondrial structure in hippocampal neurons. PLoS One 2017;12: e0168840.

46. Arrazola MS, Silva-Alvarez C, Inestrosa NA. How the Wnt signaling pathway protects from neurodegeneration: the mitochondrial scenario. Front Cell Neurosci 2015;9:166.

47. Rath E, Moschetta A, Haller D. Mitochondrial function—gatekeeper of intestinal epithelial cell homeostasis. Nat Rev Gastroenterol Hepatol 2018;15:497–516.

48. Serrat R, Lopez-Domechen G, Mirra S et al. The non-canonical Wnt/FRK pathway regulates mitochondrial dynamics through degradation of the arm-like domain-containing protein Alex3. PLoS One 2013;8:e67773.

49. Rasmussen ML, Ortolano NA, Romero-Morales AI et al. Mitochondria at the interface between dynamics in pluripotent stem cells. Genes (Basel) 2018;9:109.

50. Vlad-Fiegen A, Veronika Freytag N, Dorn S et al. The Wnt pathway target gene CCND1 changes mitochondrial localization and decreases mitochondrial activity in colorectal cancer cell line SW480. JBM 2016;04:132–43.

51. Mills KM, Brocardo MG, Henderson BR. APC binds the Miro/Milton motor complex to stimulate transport of mitochondria to the plasma membrane. MBio 2016;27:466–82.

52. Brown K, Yang P, Salvador D et al. WNT/beta-catenin signaling regulates mitochondrial activity to alter the oncogenic potential of melanoma in a PTEN-dependent manner. Oncogene 2017;36:3119–36.

53. Wen YA, Xiong X, Scott T et al. The mitochondrial retrograde signaling regulates Wnt signaling to promote tumorigenesis in colon cancer. Cell Death Differ 2019;26:1955–69.

54. Bernkopf DB, Behrens J. Feedback regulation of mitochondrial homeostasis via Wnt/beta-catenin signaling. Mol Cell Oncol 2018;5:e1458015–05.

55. Bernkopf DB, Behrens J. Feedback regulation of mitochondrial homeostasis via Wnt/beta-catenin signaling. Mol Cell Oncol 2018;5:e1458015–05.

56. Zhang Q, Wu X, Chen P et al. The mitochondrial unfolded protein response is mediated cell-non-autonomously by retromer-dependent Wnt signaling. Cell 2018;174:870–83 e17.

57. Clevers HC, Bevins CL. Paneth cells: masteros of the small intestinal crypts. Nat Rev Physiol 2013;75:289–311.

58. Rhee SG. Mitochondria: a central player in cellular stress responses in chronic inflammation. Nat Rev Mol Cell Biol 2015;16:1863:2065–71.

59. Shi M, Zhang J, Li X et al. Mitochondria-targeted delivery of doxorubicin to enhance antitumor activity with HER-2 peptide-mediated multifunctional pH-sensitive DQAsomes. Int J Nanomedicine 2018;13:4209–26.

60. Tian Y, Zhang H, Qin Y et al. Overcoming drug-resistant lung cancer by paclitaxel-loaded hyaluronic acid-coated liposomes targeted to mitochondria. Drug Dev Ind Pharm 2018;44:2071–82.

61. Yun CW, Han YS, Lee SH. PGC-1alpha controls mitochondrial biogenesis in drug-resistant colorectal cancer cells by regulating endoplasmic reticulum stress. Int J Mol Sci 2019;20: E1707.

62. Ishii I, Harada Y, Kasahara T. Reprofilin as a classical antihemorrhagic, pyrvinium pamoate, as an anti-cancer drug targeting mitochondrial respiration. Front Oncol 2012;2:137.

63. Thorne CA, Hanson AJ, Schneider J et al. Small-molecule inhibition of Wnt signaling through activation of casein kinase 1alpha. Nat Chem Biol 2010;6:829–36.
83. Erxleben A. Mitochondria-targeting anticancer metal complexes. Curr Med Chem 2019;26:694–728.
84. Li J, He X, Zou Y et al. Mitochondria-targeted platinum(II) complexes: dual inhibitory activities on tumor cell proliferation and migration/invasion via intracellular trafficking of beta-catenin. Metallomics 2017;9:726–33.
85. Nangia-Makker P, Yu Y, Vasudevan A et al. Metformin: a potential therapeutic agent for recurrent colon cancer. PLoS One 2014;9:e84369.
86. Krishnamurthy N, Kurzrock R. Targeting the Wnt/beta-catenin pathway in cancer: update on effectors and inhibitors. Cancer Treat Rev 2018;62:50–60.
87. Shang S, Hua F, Hu ZW. The regulation of beta-catenin activity and function in cancer: therapeutic opportunities. Oncotarget 2017;8:33972–89.