

Energy metabolism in neuroblastoma and Wilms tumor

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Abstract: To support high proliferation, the majority of cancer cells undergo fundamental metabolic changes such as increasing their glucose uptake and shifting to glycolysis for ATP production at the expense of far more efficient mitochondrial energy production by oxidative phosphorylation (OXPHOS), which at first glance is a paradox. This phenomenon is known as the Warburg effect. However, enhanced glycolysis is necessary to provide building blocks for anabolic growth. Apart from the generation of ATP, intermediates of glycolysis serve as precursors for a variety of biosynthetic pathways essential for cell proliferation. In the last 10-15 years the field of tumor metabolism has experienced an enormous boom in interest. It is now well established that tumor suppressor genes and oncogenes often play a central role in the regulation of cellular metabolism. Therefore, they significantly contribute to the manifestation of the Warburg effect. While much attention has focused on adult solid tumors, so far there has been comparatively little effort directed at elucidation of the mechanism responsible for the Warburg effect in childhood cancers. In this review we focus on metabolic pathways in neuroblastoma (NB) and Wilms tumor (WT), the two most frequent solid tumors in children. Both tumor types show alterations of the OXPHOS system and glycolytic features. Chromosomal alterations and activation of oncogenes like *MYC* or inactivation of tumor suppressor genes like *TP53* can in part explain the changes of energy metabolism in these cancers. The strict dependence of cancer cells on glucose metabolism is a fairly common feature among otherwise biologically diverse types of cancer. Therefore, inhibition of glycolysis or starvation of cancer cells through glucose deprivation via a high-fat low-carbohydrate diet may be a promising avenue for future adjuvant therapeutic strategies.

Keywords: Cancer metabolism; mitochondria; neuroblastoma (NB); oxidative phosphorylation (OXPHOS); Wilms tumor (WT)

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Introduction

The Warburg effect was first described by Otto Warburg in the 1920s, for which he was honored with a Nobel Prize in 1931. Warburg observed that tumor cells favor oxidizing glucose to lactate, rather than to CO₂, even with abundant oxygen present (1), which seems paradoxical at first glance. For decades it was assumed that the Warburg effect was provoked by an insufficient supply of blood vessels to the tumor, leading to hypoxia and thereby forcing the tumor cells to adjust to the oxygen shortage by up-

regulating glycolysis. However, even with ample oxygen supply, most solid tumors still show increased glycolytic activity and lowered respiration. In clinical diagnostics, the Warburg effect is exploited in the application of positron emission tomography (PET) to detect malignant tumors and metastases by virtue of their higher glucose uptake and retention compared to normal cells.

In the presence of ample glucose, glycolysis generates ATP more rapidly than oxidative phosphorylation (OXPHOS) (2). It is now accepted that enhanced glycolysis is necessary to provide building blocks for

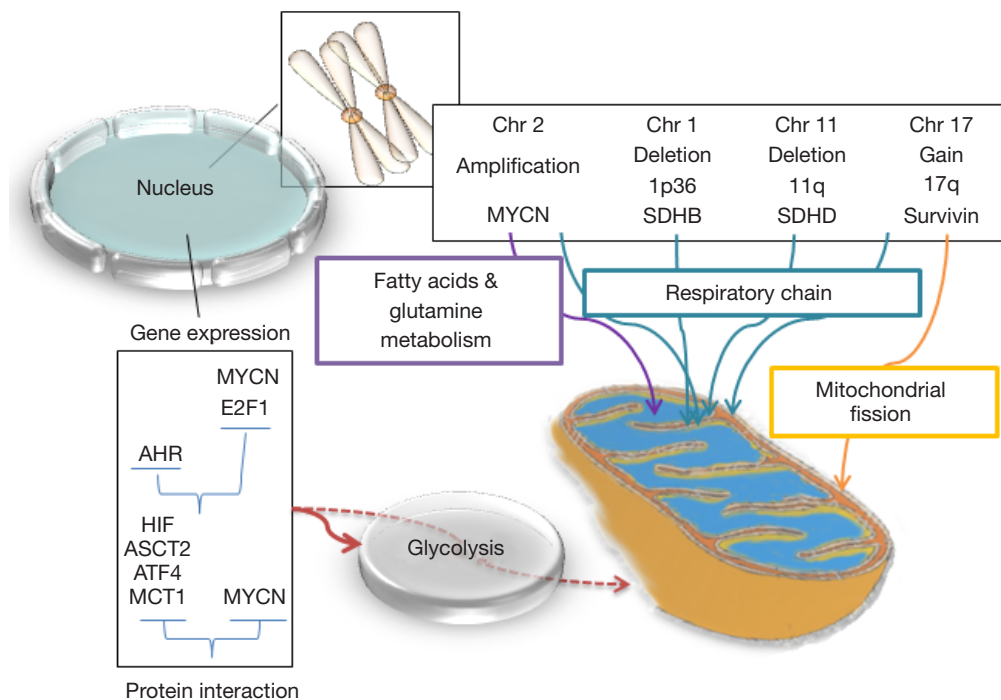


Figure 1 Some of the most common genetic abnormalities in NBs that affect different aspects of mitochondria such as morphology, respiratory chain function and metabolism. NBs frequently exhibit *MYCN* amplification, deletion of chromosome region 1p36, partial or complete loss of chromosome 11q, and gain of chromosome 17q. *SDHB* is located on chromosome region 1p36, *SDHD* on chromosome 11, both subunits of the OXPHOS complex II. Different studies have demonstrated various influences of N-Myc in correlation with other factors on mitochondrial or cytosolic metabolism in *MYCN*-amplified NBs. *MYCN* regulates gene expression and interacts with certain important factors determining the mode of energy generation (glycolysis vs. OXPHOS). *SDHB/SDHD*, succinate dehydrogenase subunit B/D; *AHR*, aryl hydrocarbon receptor; *HIF*, hypoxia inducible factor; *MCT*, monocarboxylate transporter; NB, neuroblastoma; OXPHOS, oxidative phosphorylation.

anabolic growth (3). Apart from the generation of ATP, intermediates of glycolysis serve as precursors for a variety of biosynthetic pathways necessary for cell proliferation. Thus, glucose metabolism impacts the synthesis of nucleotides, amino acids and fatty acids (2,4,5).

The bulk of cellular energy is normally produced in mitochondria, the cellular organelles that produce and regulate energy in the form of ATP through the five complexes of the OXPHOS system. Mitochondrial energy metabolism includes pyruvate oxidation, the citric acid cycle, OXPHOS and β -oxidation of fatty acids. Mitochondria additionally are associated with several vital cellular functions, including apoptosis, calcium signaling, cellular stress response, and many anabolic and catabolic pathways (6).

In the last decade several laboratories have investigated changes of the OXPHOS system in cancer. We and others were able to show that, depending on the tumor type,

cancer cells are frequently characterized by either a general low amount of mitochondria and OXPHOS (7), a specific defect of one OXPHOS complex (8-12) or a combined low level of OXPHOS complexes (13).

Also, the molecular mechanisms leading to the metabolic rewiring of cancer cells have received intense investigation. Those studies demonstrated that genetic alterations of cancer cells frequently affect oncogenes and tumor suppressor genes that regulate cellular metabolism (Figure 1).

Here we review the literature related to cancer energy metabolism in neuroblastoma (NB) and Wilms tumor (WT). NBs and WTs are the two most frequent tumors in children together accounting for nearly 15% of all pediatric cancers (14,15). They occur in early childhood and typically present as large abdominal masses closely associated with the kidneys. NBs are presumed to originate from neural crest sympathoadrenal progenitors (neuroblasts) and WTs from the primitive metanephrogenic blastema (16).

Regulation of cancer metabolism

The switch to glycolysis and lowered OXPHOS activity in cancer cells can be achieved by a broad variety of mechanisms. Here we provide an overview of the most important regulators of cellular energy metabolism and how oncogenes and tumor suppressors can interact with these regulatory pathways (Figure 1).

The most important sensor of aerobic and anaerobic conditions is hypoxia inducible factor (HIF), which is the major transcription factor responsible for gene expression changes under hypoxic conditions. Tissue hypoxia is involved in the progression and chemoresistance of many types of tumors because it affects a plethora of genes associated with metabolism, angiogenesis, cell survival and apoptosis (17). Under normoxic conditions, HIF-1 α undergoes permanent, oxygen-dependent hydroxylation, and is thus recognized and ubiquitinated by the von Hippel-Lindau tumor suppressor (VHL) and ultimately degraded. However, HIF-1 α can also be activated under normoxic, albeit pathological conditions by certain oncogenic signaling pathways (e.g., mutation of *VHL* in renal cell carcinoma). In this case a “pseudo-hypoxic” condition is induced while oxygen is present. When activated, HIF-1 α stimulates the transcription of genes encoding glucose transporters and many glycolytic enzymes and activates pyruvate dehydrogenase kinases (PDKs), which reduces the flow of glucose-derived pyruvate into the tricarboxylic acid (TCA) cycle (17). AMP-activated protein kinase (AMPK) is a crucial sensor of energy status and plays an important role in the cellular response to metabolic stress. It functions as a metabolic checkpoint, regulating the cellular response to energy availability. When cells undergo energetic stress (owing to prolonged starvation or excessive exercise), AMPK is activated in response to an increased AMP/ATP ratio. Thus, activated AMPK shifts cells to an oxidative metabolic phenotype, provokes cell-cycle arrest and inhibits proliferation (18,19). Cancer cells must escape this checkpoint to proliferate. Several oncogenes are able to suppress AMPK signaling to allow tumor cells to divide even during energetic stress conditions. Loss of AMPK leads to activation of mammalian target of rapamycin (mTOR) and subsequently HIF-1; therefore, inactivation of AMPK contributes to the switch to aerobic glycolysis (20).

The MYC family of genes includes *MYC*, *MYCN* and *MYCL* that encode, respectively, the transcription factors MYC, MYCN and MYCL. Myc transcription factors coordinate the control of cell growth, division,

and metabolism (21). *MYCs* are proto-oncogenes involved in many signal transduction pathways that are responsive to growth factors or to inputs from the cellular microenvironment. Expression of these transcription factors is deregulated in many human cancers. MYC induces an abnormal transcriptional response network, controls cell growth and proliferation, and affects different aspects of cellular metabolism by robustly activating key genes involved in mitochondrial biogenesis, glucose and glutamine metabolism, and lipid synthesis (22). When MYC is highly expressed it collaborates with HIF in the regulation of glucose transporters, glycolytic enzymes, lactate dehydrogenase A (LDHA) and PDK1 (23).

p53 is a tumor suppressor and transcription factor well known for its important roles in apoptosis, the DNA-damage response, and regulation of cellular metabolism. It activates hexokinase 2 (HK2) that converts glucose to glucose-6-phosphate, which enters either glycolysis to produce ATP, or the pentose phosphate pathway to support macromolecule biosynthesis. Under normal conditions, p53 inhibits the glycolytic pathway by supporting the expression of *PTEN* (phosphatase and tensin homolog) that inhibits phosphatidylinositol 3-kinase (PI3K). Therefore, loss of p53 is an important boost towards acquisition of the glycolytic phenotype (24). Moreover, octamer-binding transcription factor 1 (OCT1) is often overexpressed in several human cancers. OCT1 cooperates with p53 to regulate the balance between glycolytic and oxidative metabolism. OCT1 enhances resistance to oxidative stress, regulates genes responsible for up-regulating glucose metabolism, and reduces mitochondrial respiration in cancer cells (25).

Neuroblastoma (NB)

The beginnings of research on NB go back to a study of 60 cases during 1944-1970 at the M. D. Anderson Hospital and Tumor Institute (26). NB is the most frequent malignancy of the sympathetic nervous system in children, with an incidence of 1.3 cases per 100,000 children (aged 0-14 years) (27,28), accounting for approximately 15% of all cancer deaths in children (28). Histologically and genetically, NB is a heterogeneous tumor with an overall likelihood of 60% that it will metastasize (29). NBs are classified as peripheral neuroblastic tumors (pNTs) that derive from the neural crest sympathoadrenal lineage that would normally develop and differentiate into sympathetic ganglia, chromaffin cells of the adrenal medulla, and the paraganglia (28). Extensive genetic studies on NBs

uncovered a variety of genetic changes that can be broadly grouped as chromosomal aberrations and genetic mutations (Figure 1). The most frequent chromosomal aberrations are allelic loss of 1p36 and 11q (30-33) and gain of 17q (34,35). The loss of heterozygosity (LOH) of 1p36 is highly associated with *MYCN* amplification. Amplification of *MYCN* is a well-recognized genetic marker of high stage NB with poor prognosis. Somatic mutation or amplification of the anaplastic lymphoma receptor tyrosine kinase (*ALK*) gene is another frequent genetic lesion in NB (36).

Alterations of the OXPHOS system in neuroblastic tumors

NBs, like most other solid tumors, show high glucose uptake on PET examination (37,38). The Warburgian tendency to glycolysis brings about an elevated need for glucose, and excessive lactate production (6). Accordingly, NBs show a high rate of lactic acid production and a low rate of oxygen consumption, reflecting the switch from mitochondrial OXPHOS to glycolysis (39).

There is strong evidence that the Warburg effect in NBs is not caused by a low mitochondrial mass per cell. Electron microscopy showed numerous round and oval or elongated mitochondria in NB cells (40). Consistent with this observation, we recently reported equal levels of citrate synthase activity and porin expression in human NB samples and normal kidney tissue, indicating a similar mitochondrial mass (41). Furthermore, MitoTracker Green analysis indicated similar amounts of mitochondria in NBs, cerebral cortex and retinal ganglion cells (42). Strikingly, a significant reduction in mtDNA copy number and an overall reduction of OXPHOS enzyme activities were observed in human NB tissue; in comparison with cortical kidney tissue, the activity of succinate dehydrogenase (SDH; or complex II) was most affected (41). Interestingly, a similar phenotype was observed in adult renal cell carcinoma which is characterized by different genetic alterations (7).

Ganglioneuromas (GNs) and ganglioneuroblastomas (GNBs), like NBs, are classified as pNTs that originate from immature sympathetic neuroblasts. Intermixed GNBs are histologically and clinically situated between NBs and the benign GNs, while nodular GNBs are composed of nodules of NB cells in the background of intermixed GNBs. According to the Shimada classification, GNs are Schwannian stroma-dominant tumors, whereas NBs are considered as Schwannian stroma-poor neoplasias (43). Surprisingly, GNs show a severe loss of mitochondria, as

indicated by loss of porin and significant reduction of citrate synthase; therefore, their loss of all OXPHOS complexes compared to normal adrenal gland and kidney cortex tissue is likely secondary to mitochondrial loss (44).

The nexus between common genetic alterations in NB and energy metabolism

Deletions of chromosome 1p36 occur in approximately 20-40% of primary NBs. As noted above, LOH of 1p is also highly correlated with *MYCN* amplification (32,33). Succinate dehydrogenase subunit B (*SDHB*) maps to chromosome 1p36, a region of frequent LOH in NB (31). There may be a correlation between 1p36 LOH and the low activity of SDH reported by our laboratory (41). Sequencing of all four subunits of SDH and two assembly factors showed no pathological mutations in human NB samples (41). These findings are in agreement with those of two other studies, which also failed to detect pathogenic mutations in *SDHB* and *SDHD* in NB. Furthermore, epigenetic studies also could not demonstrate evidence for silencing of *SDHB* expression (45,46). However, rare cases of NB with *SDHB* and *SDHD* mutations have been reported (46-48). NB cells transfected with different *SDHB*-mutated constructs showed down-regulation of mitochondrial and cytoplasmic metabolism as well as higher growth rates (49).

Deletion of the long arm of chromosome 11 is observed in high-risk NBs with an unfavorable prognosis (33). The *SDHD* gene is located on 11q23. A significant reduction in *SDHD* mRNA expression in NBs compared to both normal fetal neuroblasts and various normal adult tissues was demonstrated (46).

Gain of chromosome 17 is also a frequent chromosomal abnormality detected in advanced-stage NBs. Two patterns of gain are seen for this chromosome: either the whole chromosome 17 or only 17q is multiplied (34). NBs with a 17q gain overexpress *BIRC5/Survivin*, an anti-apoptotic protein (50). It was reported that the survivin protein is involved in reprogramming metabolism in NB cells by inducing fragmentation of mitochondria and reducing mitochondrial respiration. Survivin shifts the energy production from OXPHOS to aerobic glycolysis through inhibition of complex I and induction of mitochondrial fission via recruitment of dynamin 1-like protein/dynamin-related protein 1 (*DNM1L/Drp1*). NB cells with elevated survivin levels showed faster glucose metabolism and higher lactate production. Furthermore, inhibition of complex

I by survivin prevented reactive oxygen species (ROS) accumulation and Forkhead box O3 (FOXO3) activity (50). Feichtinger *et al.* reported low complex I activity in NBs, although no data regarding gain of chromosome 17 were available (41).

Amplification of *MYCN* is found in half of all high-risk NBs with poor prognosis and resistance to therapy (51). Studies showed that *MYCN* is associated with regulation of energy metabolism through direct or indirect activation of genes involved in glycolysis, glutamine metabolism, fatty acids metabolism and mitochondrial function (52-54). Amplification of *MYCN* supports the Warburg effect in NBs by activating the transcription of several glycolytic genes. Knock-down of *MYCN* expression in *MYCN*-amplified NB cell lines caused down-regulation of glycolytic metabolism. Depletion of *MYCN* led to lower expression of the genes encoding glucose transporter 1 (*GLUT1*), *HK2*, *PDK1*, phosphoglycerate kinase 1 (*PGK1*), aldolase A (*ALDA*), and *LDHA* and leads to decreased proliferation of *MYCN*-amplified NBs (52).

Interestingly, mitochondrial inhibition by metaiodobenzylguanidine (MIBG) caused destabilization of *MYCN* and *MYC* in *MYCN*-amplified and non-*MYCN*-amplified NB cells and suppressed NB cell growth (55). MIBG also reduced the ATP to ADP ratio in NB cells (56).

Abundant glutamine is necessary for the TCA cycle in *MYCN*-amplified NB. Deprivation of glutamine led to depletion of TCA-cycle intermediates (54). During anaplerosis, mitochondrial glutamine metabolism can replenish oxaloacetate (OAA) for the TCA cycle (57). Provision of sufficient levels of glutamine for NB cells relies on activation of amino-acid transporter ASCT2, a glutamine transporter. High expression of *MYCN*, *ATF4* and *ASCT2* was observed in *MYCN*-amplified NB. The elevated level of *ASCT2* expression observed in high-stage NB correlates with *MYCN* and *ATF4* expression that promotes aggressive NB progression. Knockdown of *MYCN* led to a significant reduction in *ASCT2* abundance (54).

The expression of the aryl hydrocarbon receptor (AHR), a receptor for dioxin-like compounds, correlates with a higher degree of differentiation of NBs. An inverse correlation between AHR and *MYCN* levels was reported (58). Members of the E2F family are transcription factors that regulate cell-cycle progression and influence *MYCN* expression in NBs (59). Ectopic expression of AHR correlates with down-regulation of *MYCN* and *E2F1* expression in NB cells. Silencing of *AHR* enhanced the expression of both *E2F1* and *MYCN* in NB cells. It is

proposed that AHR is an upstream regulator of *MYCN* expression (58). It was further suggested that AHR and *E2F1* are involved in mitochondrial function. Interaction between AHR and *ATP5 α 1*, a subunit of complex V, was reported, from which the authors hypothesized that AHR contributes to homeostasis of mitochondrial function and cellular energy metabolism (60). Knockdown of *E2F1* in HeLa cells produced highly elevated mRNA levels of genes involved in mitochondrial biogenesis (61). Concurrent findings also support a role for *E2F1* in regulating energy homeostasis and mitochondrial function: *E2F1* causes a shift from oxidative to glycolytic metabolism under stress conditions (62).

The monocarboxylate transporter (MCT) family member *MCT1* can transport both lactate and pyruvate. High activity of *MCT1* was correlated with *MYCN* amplification in NBs. Inhibition of *MCT1* causes acidosis and a significant reduction in viability of NB cells (63).

HIF-1 α was shown to be expressed in both *MYCN*-amplified and non-amplified NBs. It seems *HIF-1 α* in association with *MYCN* fortifies aerobic glycolysis in NBs. Knockdown of *HIF1- α* selectively decreased the expression of genes encoding *GAPDH*, *HK2*, *PDK1*, *ALDA* and *LDHA* in NBs. Concurrent knockdown of both *MYCN* and *HIF-1 α* further reduced *HK2* and *LDHA* expression (52).

Other regulators of metabolism in NB

Transient receptor potential cation channel, subfamily M, member 2 (TRPM2) is an ion channel that is frequently expressed in cancers. Full-length TRPM2 (TRPM2-L) protected NBs from moderate oxidative stress. The other form of TRPM2 is a dominant-negative short isoform (TRPM2-S). NBs which expressed TRPM2-S showed a significant reduction in tumor growth compared to TRPM2-L-expressing NBs. Significantly reduced expression of *HIF-1/2 α* was detected in TRPM2-S-expressing NB cells. Interestingly, the target proteins regulated by *HIF-1/2 α* in mitochondria (e.g., *BNIP3*, *NDUFA4L2*, *COX4.1*, and *COX4.2*) were significantly down-regulated in these cells (64).

It was demonstrated that *HIF-2 α* also can play an indirect role in NB metabolism. Over-expressed *HIF-2 α* but weak *HIF-1 α* expression was detected in well-vascularized areas of NBs (65,66). Vascularization, which enables access to more oxygen and metabolites like glucose, glutamine, etc., is necessary for tumor cells. Moreover, blood vessels help tumor cells to get rid of metabolic waste products.

Contingency theories

Accumulation of succinate, the substrate of SDH, correlates with stability of HIF-1 α via inhibition of the HIF-alpha prolyl hydroxylases activity (67). Therefore, it is possible that a low level of SDH activity may intensify the hypoxia response and induce reprogramming of metabolism via HIF-1 α in NBs. It was shown that hypoxia induces dedifferentiation and a shift to an immature neural crest-like phenotype in NBs, a change associated with greater aggressiveness and a higher potential to metastasize (68).

Wilms tumor (WT)

WT, also known as nephroblastoma, is a pediatric cancer that affects the kidneys (69). The name derives from Dr. Max Wilms, the German surgeon who first described it. Most WTs are unilateral; both kidneys are affected in only 5-6% of cases (70,71). WT usually presents as a single mass encapsulated in fibrous tissue, and thus is well separated from the adjacent kidney tissue (71).

WTs are believed to arise from the malignant transformation of renal stem cells that abnormally persist after embryogenesis and maintain embryonic differentiation capacity. WTs are usually triphasic, which implies they are composed of three main types of tissue: mesenchyma, blastema and epithelium. The mesenchymal tissue is comprised of immature embryonal mesenchyme, but may also contain more differentiated structures (e.g., skeletal muscle, adipose tissue). The blastemal tissue consists of sheets of undifferentiated cells. Finally, WT contains epithelial structures including immature glomeruloid bodies or tubules (71). The overall survival rate of patients with WTs is greater than 90% (15). WTs are histologically divided into two main groups: anaplastic WTs (approximately 10%), characterized by large cells with large hyperchromatic nuclei, multipolar mitotic figures associated with poor response to chemotherapy and lower survival rate; and non-anaplastic WTs that have more favourable survival outcomes (70).

WTs are characterized by genetic abnormalities involving tumor suppressor genes (e.g., *WT1* and *TP53*), oncogenes (e.g., *HIF-1* and *MYC*), and other genes related to the WNT signaling pathway. Recently, somatic mutations in *DROSHA* and *DICER1* were identified in 12% of WTs, two factors regulating miRNA expression (72,73). Furthermore, several genetic syndromes are associated with an increased risk of developing WT. The most common of these are:

Wilms-aniridia-genitourinary-mental retardation (WAGR) syndrome, due to deletion of chromosome 11p13 in a region containing the *WT1* and *PAX6* genes (74,75); Denys-Drash syndrome (DDS), associated with constitutional abnormalities of the *WT1* gene (76,77); Beckwith-Wiedemann syndrome (BWS), a pediatric overgrowth disorder with a predisposition to develop embryonal malignancies (78), caused by mutation or deletion of imprinted genes on chromosome 11 (79); and Fanconi anemia (FA), a rare recessive disorder caused by genetic defects in a number of proteins involved in, and associated with chromosomal fragility and genomic instability. There are different subgroups of FA; in FA complementation group D1, a severe form of the disease, biallelic mutation of *BRCA2* is causative, and 63% of children with this genotype will develop WTs by the age of 6.7 years (80,81).

Alterations of the energy metabolism in WT

The role of fluoro-D-glucose (FDG)-PET in WTs has not been well established (82). However, there are some case reports demonstrating the WTs can be monitored by PET examination (83). In classical triphasic WT, the epithelial, blastemal and stromal elements differ in terms of mitochondrial energy metabolism. Normal mitochondrial mass was reported for the blastemal and epithelial areas. In contrast, the stroma regions showed severe loss of mitochondria compared to normal kidney tissue, resulting in loss of all OXPHOS complexes, as indicated by low porin expression and citrate synthase activity. The mtDNA copy number was also reduced in WT, which can partly explain the reduction in OXPHOS enzymes (84). These observations are supported by ultrastructural studies, which showed that mesenchymal cells contain few mitochondria, whereas primitive epithelial cells possess numerous mitochondria (85).

In agreement with these data, Hammer and colleagues detected a general decrease of mitochondrial metabolism in WTs. By mass spectrometric analyses of formalin-fixed paraffin-embedded tissue sections, they showed that proteins involved in OXPHOS and other mitochondrial functions (OXPHOS complex components and TCA cycle enzymes) were present at lower levels in WT samples compared with normal kidney tissue. In general, many other metabolic proteins were found to be decreased in WTs compared to normal tissue, for example aldehyde dehydrogenases and proteins related to propanoate and butanoate metabolism, long-chain fatty acid metabolism, and degradation of the

branched-chain amino acids valine, leucine and isoleucine. However, because the tissue samples were obtained after chemotherapy treatment, it is unclear if these changes are also present in primary, untreated WT (86).

In WTs, HIF-1 α has been found to be expressed at higher levels compared to normal kidney. Moreover, localization of HIF-1 α in the tumor tissue is predominantly nuclear, whereas in normal kidney it is cytoplasmic (87,88). As previously mentioned, HIF-1 α plays an important role in tumor development in different ways. For example, HIF-1 α increases the expression and activity of many different glycolytic protein isoforms, including the transporters GLUT1 and GLUT3 that transport glucose across the plasma membrane, enhancing tumor glycolytic flux. HIF-1 α seems also to be involved in the modulation of mitochondrial function and oxygen consumption by inactivating the pyruvate dehydrogenase (PDH) complex in some tumor types (89). Therefore, overexpression of HIF-1 α and its nuclear localization, together with the reductions in mitochondrial biogenesis and/or OXPHOS activity found in WTs, suggest that these types of tumors are characterized by the metabolic switch to aerobic glycolysis.

Interestingly, there are no further studies available which have investigated the metabolism of WT in detail regarding the metabolic pathways known to be altered in a range of cancers (see "Regulation of cancer metabolism"). There is only indirect evidence from the genes altered in WT that there are alterations of cell metabolism.

The nexus between genetic alterations in WT and energy metabolism

About 5-6% of WTs present with *TP53* mutations. Interestingly, *TP53* mutations are mostly found in anaplastic WT, indicating an association with a more aggressive tumor phenotype (58,90,91). *TP53* is a tumor suppressor gene involved in the development of many types of cancer. It plays a role in the regulation of many genes, including acting as a transcriptional repressor of GLUT1, and therefore it is important in glycolysis regulation. In anaplastic WT a stronger expression of GLUT1 compared to non-anaplastic WT was observed (92). Moreover, as previously mentioned, GLUT1 is stimulated by HIF-1 α . Since p53 mediates the repression of HIF-1 α , HIF-1 α and p53 seem to cooperate in establishing the Warburg effect in anaplastic WTs and thereby could be responsible for the development of an aggressive phenotype. Interestingly, Zawacka-Pankau et al. showed that pharmacological re-activation of p53

in tumor cell lines and xenografts decreases the levels of HIF-1 α , both in normoxic and hypoxic conditions, and they also found that p53 binds to the promoter of *HIF-1 α* , inhibiting its expression. Moreover, they showed that the pharmacological re-activation of p53 is able to down-regulate metabolic genes, including the gene responsible for the transcription of GLUT1, confirming the interdependence of p53 and HIF-1 α in the regulation of the Warburg effect in cancer cells (93). The morphology and physiology of WTs resemble those of the developing kidney (94). For example, the WNT pathway is required during embryonic development to maintain the pluripotency and plasticity of human embryonic stem cells (71). Activation of the WNT/ β -catenin pathway is an important hallmark of stemness of cancer stem cells, and β -catenin mutations have been found in 15% of WTs (95,96). In WTs, mutations affecting *CTNBN1*, the gene encoding β -catenin, usually result in constitutive activation of the WNT/ β -catenin signaling pathway (95). In one study, the authors reported that at least 50% of tumors with *WT1* mutations also had a β -catenin mutation (97,98).

Recently, it was shown that WNT signaling works in a context and tissue specific manner. So far 19 *WNT* genes were identified. WNT signaling can be divided into a canonical and a non-canonical pathway, which is β -catenin independent. In a recent paper it was shown that WNT3A (non-canonical) can activate mitochondrial biogenesis and oxygen consumption in mouse myotubes. WNT5A suppressed this action (99). In contrast, WNT3A was shown to increase glycolytic capacity during osteoclast differentiation (100). In addition, WNT5A expression correlated with good or bad prognosis depending on the analyzed tumor type (101-104). Activation of the canonical WNT/ β -catenin signaling was shown to increase aerobic glycolysis through suppression of mitochondrial respiration (105,106).

An important downstream target of the WNT/ β -catenin pathway is *MYC*, a proto-oncogene that functions as transcription factor for many genes involved in cell cycle. The *MYC* and *MYCN* genes have similar 5' noncoding exons and extensive homology in the coding regions, and they are both overexpressed in many WTs (107,108). As *MYC* promotes glycolysis and energy production and is a key regulator of glutaminolysis in transformed cells (109), and *MYCN* in association with HIF-1 α increases the transcription of glycolytic enzymes (52), these observations indicate that the WNT signaling pathway influences cancer cell metabolism.

Expression of *IGF-2* is increased in 69% of WTs (110).

IGF-2 is an imprinted gene normally expressed only from the paternal allele. In sporadic WTs, this gene often exhibits bi-allelic expression (111). An increase of IGF-1 or IGF-2 leads to activation of the insulin signaling pathway. A cascade of events leads to the activation of the PI3K-mitogen activated protein kinase (MAPK)-Akt signaling pathway that results in an increase in glucose uptake and metabolism. Furthermore, activation of Akt signaling via down-regulation of PGC-1 α decreases mitochondrial biogenesis (6). *WT1*, located on chromosome 11p13, plays an important role in genitourinary development, encoding a zinc finger transcription factor and acting as a tumor suppressor. *WT1* loss-of-function mutations occur in 10-20% of WT cases (95,97). *WTX*, located on chromosome Xq11.1, was also found to be involved in WT development. The prevalence of *WTX* mutations is approximately 18% in WTs (112). Both *WT1* and *WTX* mutations cause dysregulation of the WNT/ β -catenin pathway, and consequently abnormal cell proliferation (97,112).

Mutations in the *WT1* gene can affect E-cadherin expression. It can induce epithelial differentiation during mesenchymal-epithelial transition and E-cadherin expression in the embryonic kidney (113). Loss or reduction of mtDNA results in hypermethylation of the E-cadherin gene promoter (114). When the tumor cell lines, LNrho0-8 (derived from the LNCaP prostate cancer cell line) and MCFrho0 (derived from the MCF-7 breast cancer cell line) were depleted of mtDNA by treatment with ethidium bromide, the cells lost their epithelial features and gained a mesenchymal phenotype. Furthermore, repression of E-cadherin and induction of vimentin expression were observed. Down-regulation of E-cadherin in LNrho0-8 and MCFrho0 cells was shown to be due to hypermethylation of the E-cadherin promoter region (115). We propose that the reduction of mtDNA copy number leading to a decline in OXPHOS activity in WT induces epigenetic changes in nuclear DNA, influencing EMT. Because only 10-15% of sporadic (116,117) WTs carry a *WT1* mutation, but many have low mtDNA levels, low mtDNA and OXPHOS might be the main trigger for low E-cadherin expression.

It has been shown in human embryonic kidney cells and murine mesonephric cells, that inducing or repressing *WT1* expression affects the expression of genes involved in the synthesis of cholesterol and fatty acids (118). The mevalonate pathway is involved in the production of cholesterol, vitamin D, steroid hormones and bile acids. Transcription of these genes needs activation by sterol regulatory element-binding proteins (SREBPs). Rae

et al. hypothesize that WT1 interacts with SREBP to block transcription of mevalonate pathway genes (118). Therefore, it is reasonable to speculate that, in tumors where *WT1* is mutated, the synthesis of cholesterol and other fatty acids is increased. This would offer an advantage to the tumor since these are building blocks for cellular membranes and thus important for cell proliferation.

Therapeutic options

As previously mentioned, NB and WT cells demonstrate high glucose uptake and preferentially ferment glucose to lactate instead to CO₂ and H₂O (OXPHOS), which shows their dependency on glycolysis (83). In addition, it was discussed that NBs and WTs have OXPHOS deficiencies. All this evidence supports the idea that targeting the Warburg effect may be a promising therapeutic strategy against NB and WT. Some results that support this idea in NBs are presented next.

Inhibition of glycolysis causes a decrease in NB cell proliferation *in vitro*, underlining the reliance of NB cells on glycolysis. A xenograft study showed that 3-BrOP, a novel inhibitor of glycolysis, was able to decrease NB mass by 78% in mice (39). Dichloroacetate (DCA) is a drug which indirectly activates PDH, causing a shift in pyruvate metabolism from lactate to the TCA cycle. It was shown that DCA is able to reduce lactate production and enhance oxygen consumption in NB cells and inhibits proliferation of highly malignant NB cells (119,120). Inhibition of LDHA, the enzyme that metabolizes pyruvate to lactate, was also able to suppress the tumorigenic capacity of NBs *in vivo* (52). Depriving NBs of glucose had detrimental effects on them. Glucose-free medium supplemented with the ketone bodies, acetoacetate and hydroxybutyrate reduced the viability of NB cells (121). Also, a higher level of NB cell differentiation was observed in low-glucose medium (122). Stimulating NB cells to differentiate by treating them with differentiating agents such retinoic acid (RA) or 2-O, 3-O-desulfated heparin (ODSH) had a suppressive effect on tumor growth and metastasis (123,124). As NB cells are sensitive to glucose depletion, a high-fat low-carbohydrate ketogenic diet can be considered a non-toxic therapeutic strategy. On a ketogenic diet, NB cells would be forced to obtain their energy from fatty acids through OXPHOS. However, since NBs are unable to respire due to mitochondrial defects, they might be selectively killed by a ketogenic diet.

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Footnote

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