Chapter

Metabolic Features of Neurofibromatosis Type 1-Associated Tumors

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Abstract

Rewiring cellular metabolism is a key hallmark of cancer. Multiple evidences show that alterations in various metabolic circuits directly contribute to the tumorigenic process at different levels (e.g. cancer initiation, metastasis, resistance). However, the characterization of the metabolic profile of Neurofibromatosis type 1 (NF1)-related neoplastic cells has been only partially elucidated both in benign neurofibromas and in malignant peripheral nerve sheath tumors (MPNSTs). Here, we illustrate the state of the art on the knowledge of the metabolic features of tumors related to NF1 and discuss their potential implications for the development of novel therapeutic perspectives.

Keywords: NF1, metabolism, mitochondria, chaperones, sirtuins, MPNST, neurofibroma, glucose, glutamine, PET

1. Introduction

Neurofibromatosis type 1 (NF1) is a genetic multisystem disorder that predisposes to the onset of several tumor types and is characterized by a number of clinical manifestations encompassing café au lait macules in the skin, iris hamartomas (Lisch nodules), cognitive deficits, axillary or groin freckles, bone deformities, optic gliomas and Schwann cell neoplasms called neurofibromas. The presence of two or more of these clinical features is used as consensus diagnostic criteria for NF1 [1].

NF1 is inherited in an autosomal dominant way when inactivating mutations occur at the *NF1* locus that encodes for the Ras-GTPase activating protein (Ras-GAP) neurofibromin. Complete loss of neurofibromin activity, caused by second hit mutations, leads to hyperactivation of Ras signaling and tumor onset. The tumor type that hallmarks this genetic disease is neurofibroma, a benign neoplasm affecting peripheral nerves. Plexiform neurofibromas (PNs) involve perineural sheaths of nerve bundles and may occasionally transform into malignant peripheral nerve sheath tumors (MPNSTs), highly aggressive sarcomas endowed with a dismal prognosis. MPNSTs are currently untreatable, while only recently an inhibitor of MEK, a downstream effector of Ras signaling, has been approved for pediatric inoperable neurofibromas [2].

PN monitoring is critical for managing tumor progression and early malignancy diagnosis. To this purpose, imaging tools are extremely important in identifying suspicious lesions, and an increase in the avidity for the radioactive tracer

^{18}F-fluorodeoxyglucose (FDG) during Positron Emission Tomography (PET) scans is a critical indication of malignant progression [3–5]. This increase in glucose uptake denotes that some neoplastic cells inside the PN mass are undergoing a metabolic rewiring. Glucose is used by various intracellular metabolic pathways for the overall energetic and anabolic needs of highly proliferative cells, as it provides them with several advantages, such as induction of nucleotide and amino acid biosynthetic pathways that stem from glycolysis intermediates, as well as enhancement of anti-oxidant defenses by boosting the pentose phosphate pathway [6]. Moreover, glycolysis induction is often accompanied by a repression in cellular respiration, *aka* oxidative phosphorylation (OXPHOS), making neoplastic cells less dependent on oxygen, as its availability can often be scarce in a growing neoplastic mass that is poorly vascularized [7]. The characterization of tumor metabolic features has gained increased attention in the attempt of identifying crucial regulators of metabolism that could be exploited as pharmacological targets.

2. Metabolic features of NF1 patients

Several indications suggest that dysregulation of Ras signaling in NF1 has metabolic effects. Indeed, metabolic alterations have been identified in NF1 patients at the systemic level (**Figure 1**). For instance, in fasting conditions they show a glucose level in the blood that is lower than in control people [8] and display an increased insulin sensitivity [9] that makes them less prone to diabetes mellitus development [10]. This could be caused by a general imbalance in the levels of several hormones, including lower levels of leptin and visfatin and higher adiponectin in NF1 patients with respect to control subjects. It remains to be explained the mechanistic connection between heterozygous loss of neurofibromin and these metabolic changes, confirmed in a large cohort of patients [11]. Moreover, NF1 individuals show reduced cerebral glucose metabolism, specifically in the thalamus [12]. Altogether, these observations put forward the hypothesis that neurofibromin haploinsufficiency may have systemic effects in overall glucose utilization. Thalamic glucose hypometabolism could be related to the neurological symptoms of NF1 (e.g. cognitive impairment). By using NF1 animal models it was also proposed that other dysmetabolic traits, such as disarrangements in neuronal usage of glutamate, γ-amino butyric acid (GABA) and dopamine, could be connected to the deficits in spatial learning, memory and attention observed in patients [13–15].

Changes in the levels of these neurotransmitters could affect the activity of several ion channels linked to the neurologic phenotype of NF1. For instance, augmented activity of voltage-gated sodium and calcium channels in sensory neurons dictates increased excitability and firing properties and underlies heightened pain sensations in NF1 patients [16]. In addition, changes in ion channel properties have repercussions on non-neuronal cells in NF1 and may participate in the overall alteration of ion homeostasis, as for Ca^{2+} signaling, which is altered in NF1 keratinocytes [17]. Ca^{2+} is a highly compartmentalized ion, and its mobilization has the capability of tuning a variety of cellular processes connected to mitochondrial metabolism and cell death pathways. Whether these Ca^{2+} alterations in neurofibromin haploinsufficient cells install adaptations that are relevant also in NF1-related tumors is an intriguing possibility.

At the muscular level, NF1 children may display reduced muscle function, which has been related to a role of neurofibromin in regulating fatty acid metabolism in this tissue [18]. Muscle specimens from limb-specific Nf1Prx1$^{-/-}$ conditional knockout mice show a 10-fold increase in muscle triglyceride content, upregulation in the activity of oxidative metabolism enzymes and increased expression of

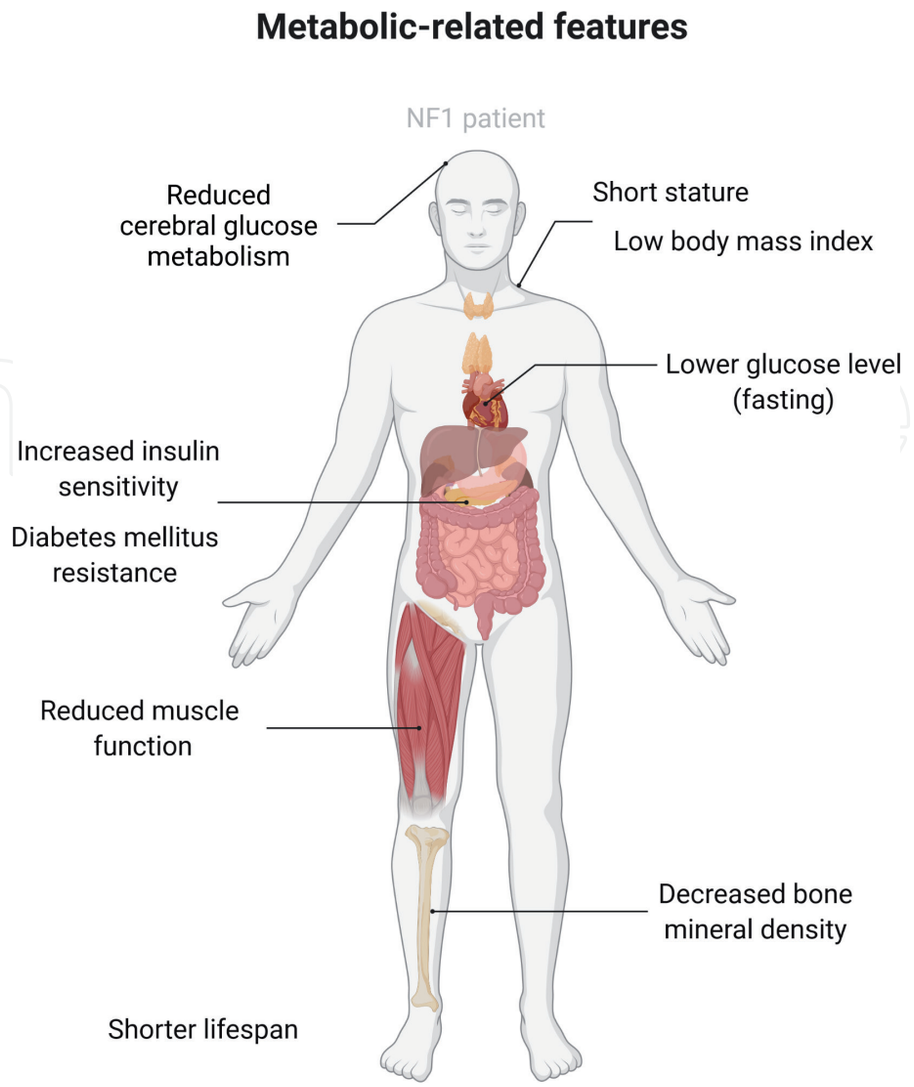

Figure 1.
The multisystem metabolic phenotype of NF1 disease. A NF1 patient is depicted with the most common metabolic-related features.

fatty acid synthase and of the hormone leptin, whereas the expression of a number of fatty acid transporters is decreased. This genetic NF1 mouse models has shown that a lipid storage disease phenotype may underlie muscle weakness in NF1, thus displaying commonalities with the lipid storage myopathies (LSMs), which also present with progressive muscle weakness and muscle lipid accumulation, and may occasionally be treated with high dose L-carnitine supplementation [19]. Nf1 null muscle specimens are enriched in long chain fatty acid (LCFA) containing neutral lipids, such as cholesterol esters and triacyl glycerides, suggesting impaired LCFA metabolism [20]. Thus, $Nf1Prx1^{-/-}$ mice recapitulate the human NF1 myopathy and lipid storage excess inside muscle fibers, and a dietary intervention of reduced LCFAs and enrichment of medium-chain fatty acids with L-carnitine effectively rescues lipid accumulation and muscle weakness in knockout mice. These data link NF1 deficiency to fundamental shifts in muscle metabolism, and provide strong proof of principle that a dietary intervention can ameliorate muscle symptoms. On the same path, pharmacological intervention with the MEK inhibitor PD0325901 in pregnant mice is able to rescue body weight loss and lipid accumulation in the $Nf1MyoD^{-/-}$ progeny, suggesting a potential mechanism underlying the NF1-Ras-MAPK dependency of altered fatty acid metabolism [21]. Furthermore, a recent work has highlighted the requirement of neurofibromin for postnatal muscle growth and metabolic homeostasis [22].

In NF1 patients, skeletal problems including scoliosis, tibial pseudo-arthrosis and short stature are also common. Bone dysplasia is considered linked to mineralization defects and is a generalized metabolic bone disease [23]. Indeed, NF1 patients display a decreased bone mineral density, low levels of serum 25-hydroxy vitamin D3, increased osteoporosis and fracture risk [24]. Whether these systemic metabolic characteristics (*i.e*. increased glucose utilization and reduced fat depot mass) could affect the timing and type of tumor manifestations remains a puzzling issue. Neurofibroma onset and growth are accelerated by the heterozygous condition of the tumor microenvironment. Similarly, it could be envisioned that circulating factors determined by the peculiar metabolism of NF1 also participate in determining the extent of cancer predisposition. Moreover, given the sophisticated regulation and adaptability of human metabolism to external factors, the understanding of its potential involvement in NF1-related tumorigenesis may shed light on the patient-to-patient variability in the tumor burden of this disease.

Take home message. Altogether, these reports underline that NF1 has multisystem effects from the metabolic point of view. Recently, some of the metabolic and morphologic features of humans with NF1 have been fully recapitulated by Nf1 heterozygous mouse models of the disease [25].

3. Metabolic adaptations of NF1-related tumors

One of the most worrisome features of NF1 disease is the increased susceptibility of patients to several neoplasms. Beside the presence of neurofibromas, benign tumors that hallmark this disorder, gliomas, hematological neoplasms, breast cancer, pheochromocytomas, gastrointestinal tumors (GISTs) and MPNSTs may develop throughout lifetime. Following the loss of the tumor suppressor gene neurofibromin and the subsequent activation of the Ras pathway, several intracellular signaling cascades are rearranged and impact on cellular processes relevant to cancer progression (e.g. survival, growth, cell death, metabolism). Beside this network of deregulated pathways inside the tumor cell, a variety of inter-cellular signals are altered by neurofibromin haploinsufficiency. Neurofibromas show a highly heterotypic microenvironment composed mainly by mast cells, macrophages and fibroblasts, and neoplastic growth depends on the complex interplay between these cell types (**Figure 2**). For instance, the KIT growth factor is secreted by NF1 null Schwann cells and acts as a chemo-attractant for NF1 heterozygous mast cells. In turn, mast cells produce TGFβ, stimulating heterozygous fibroblasts to increase production of collagen and of other extracellular matrix (ECM) proteins. Mast cells also produce heparin, vascular endothelial growth factor (VEGF) and matrix metalloproteases (MMPs), which promote tumor vascularization and invasiveness. Aberrantly proliferating Schwann cells secrete colony-stimulating factor (CSF1), thereby recruiting macrophages that sustain tumor progression.

Apart from regulating survival and proliferation, some of these alterations in signal transduction can also directly affect cellular metabolism. Indeed, RAS signaling promotes oncogenic metabolism by coordinating numerous metabolic processes including lipid, nucleotide, and glycolytic pathways (**Figure 2**). Specifically, upregulation of the Ras pathway sustains a glycolytic and glutaminolytic metabolism by MYC induction, allowing cancer cells to preferentially use glucose and glutamine for anabolic purposes. This is accompanied by a decrease in OXPHOS that is characterized by blunted TCA cycle and reduced mitochondrial respiration. Ras downstream pathways, such as the mTOR signaling, also affect lipid and nucleotide synthesis for anabolic demands [26, 27].

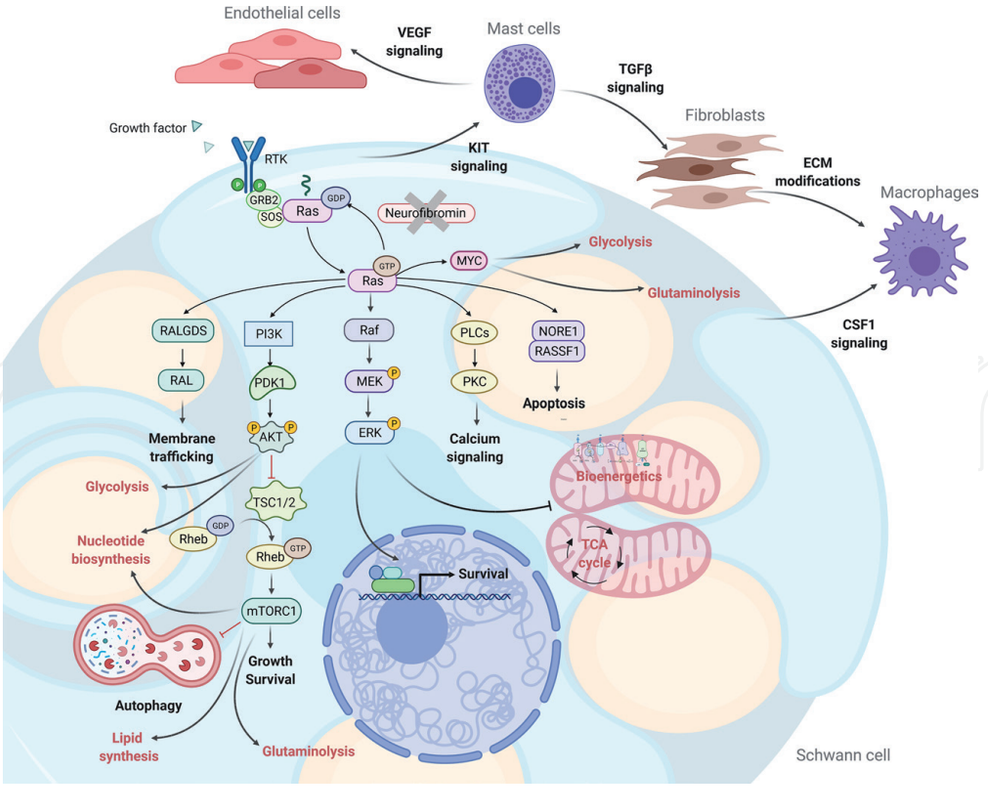

Figure 2.
Impact of neurofibromin loss on intra/intercellular signaling and metabolic pathways. Signaling cascades within Schwann cells and in the heterozygous microenvironment are highlighted in black. Metabolic pathways altered following neurofibromin loss are depicted in red.

Several metabolic circuits converge on mitochondria, which are considered the powerhouse of the cell. They are in charge of energy supply and actively sustain biosynthetic pathways mandatory for cell replication. Moreover, mitochondria are involved in cell death signaling and contribute to oxidative stress regulation. Changes in several mitochondrial functions have been linked to the pro-neoplastic dysregulation of many fundamental biological processes, including a variety of bioenergetic circuities [28].

Tumor metabolism refers to a plethora of cancer features, spanning from the way neoplastic cells take up and utilize nutrients for growth and replication, to the diverse communication modes they establish with the neighboring cells. Altogether, these metabolic adaptations during cancer initiation and progression render aberrant cells capable of circumventing nutrient and oxygen shortage conditions that they may encounter, and often affect and constrain the behavior of the surrounding microenvironment [29].

So far, targeting strategies against cancer mainly rely on specifically blocking molecular signals that promote cell proliferation, hinder cell death, modulate the immune response or enhance angiogenesis and cell survival. However, most of these signaling pathways are either redundant or essential in healthy tissue making these types of target therapies challenging. A further strategy is to hit key metabolic transformations that occur in cancer cells, whereby the metabolic adaptations to hypoxic conditions seem to be specific for cancer cells, shared in many tumor types and required for neoplastic growth.

3.1 Mitochondrial respiration

Although the metabolic scenario of NF1 mutant cells is poorly defined, some bioenergetic alterations are starting to surface. For instance, respiratory complex II,

aka succinate dehydrogenase (SDH), is a crucial metabolic enzyme at the crossroad between OXPHOS and Krebs cycle that is repressed in NF1-related tumor cells in an ERK-dependent manner following neurofibromin loss; this metabolic rewiring is compensated by an increased glycolytic pathway [30]. In detail, hyperactivation of the mitochondrial branch of Ras/ERK signaling causes phosphorylation of the mitochondrial chaperone TRAP1. Its consequent activation inhibits SDH enzymatic activity, triggering intracellular accumulation of the oncometabolite succinate that in turn stabilizes the pro-neoplastic transcription factor HIF1α. Importantly, genetic ablation of TRAP1 inhibits tumor growth [31]. Taken together, these data indicate that TRAP1 mediates a pseudo-hypoxic signaling, as it orchestrates a HIF1α-dependent program that is crucial in the neoplastic process and boosts tumor growth independently of environmental oxygen tension. Moreover, it was recently demonstrated that TRAP1 also executes the hypoxic response, as it is a transcriptional target of HIF1α induced in KRAS-dependent models of carcinogenesis, such as pancreatic adenocarcinoma, with a crucial role in handling the cell bioenergetic response to oxygen paucity [32]. In specific cases of familiar cancers (*i.e*. in the hereditary paraganglioma-phaeochromocytoma syndrome, HPGL/PCC), succinate increases following inactivating mutations of SDH subunits. In these settings, in addition to inducing HIF1α, high levels of succinate can impinge on cell epigenetics by inhibiting α-ketoglutarate dependent dioxygenases, such as histone and DNA demethylases, further contributing to neoplastic growth [33]. Therefore, it can be envisioned that similar complex changes in the epigenome landscape occur upon TRAP1-mediated SDH inhibition in NF1-related tumor cells.

As a consequence, pharmacological inhibition of TRAP1 has been proposed as an anti-neoplastic approach for MPNST and other tumor types. Recently, the identification of highly selective TRAP1 allosteric inhibitors has shown promising results, ablating *in vitro* tumorigenesis [34, 35]. Previous targeting of the HSP90 family of chaperones, to which TRAP1 belongs, has been pursued with the drug IPI-504 which, in combination with the mTOR inhibitor rapamycin, cooperates in the growth repression of NF1 mutant cancer cells [36]. Here, strong ER stress drastically represses cancer growth. Given the intense molecular crosstalk between ER and mitochondria and their coordinated regulation of Ca^{2+} homeostasis, it could be envisaged that the interplay between ER and mitochondria is crucial in the growth of NF1 deficient cells. Indeed, yeast synthetic lethality screens have identified Y100 as a molecule capable of interfering with mito-ER homeostasis, thus revealing crucial metabolic vulnerabilities of the yeast cells null for the homolog of NF1, called IRA2 [37].

Another report describes that neurofibromin-deficient cells display a decrease in the activity of NADH dehydrogenase, *aka* the first respiratory complex, with a consequent unbalance in NAD^+/NADH ratio [38]. This metabolic alteration negatively impacts on the activity of mitochondrial sirtuins, specifically SIRT3. SIRT3 reactivation through NAD^+ precursor supply or genetic manipulation impairs tumorigenesis of neurofibromin-deficient cells and synergize with TRAP1 ablation in repressing MPNST growth in xenografts by preventing HIF1α stabilization. Furthermore, the repressed expression of several subunits of the NADH dehydrogenase respiratory complex I, one of the main ROS producers in mitochondria, renders neurofibromin-deficient cells more resistant to pro-oxidant drugs acting through complex I-mediated ROS increase.

Take home message. Altogether, these data indicate that NF1-related tumors display a pseudo-hypoxic signature that contributes to tumor proliferation and transition towards malignancy. Indeed, neurofibromin inactivation occurs in certain cancers through hypoxia-induced degradation, independently of *NF1* gene mutations [39]. These data suggest that the hypoxic response might affect the Ras/ERK signaling pathways downstream to neurofibromin loss and its genetic

inactivation installs a hypoxic-like response that may provide cells with an equipped and prompt response to any possible drop in oxygen availability.

3.2 Glutamine metabolism

As already shown for several cancers, NF1 null cells are highly sensitive to glutamine deprivation, and glutaminase (GLS) inhibitors such as BPTES (bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide 3) or CB-839 have been proposed as antineoplastic agents in the context of NF1-associated neoplasms [40]. Glutamine is one of the most abundant intracellular amino acids and fuels several biosynthetic pathways by providing carbons to TCA cycle intermediates, glutathione, fatty acids, and nucleotides. Pharmacological GLS inhibition causes a shortage in multiple TCA cycle intermediates, among which α-ketoglutarate, succinate and fumarate.

Phase II Basket Trial of Glutaminase Inhibitor (BeGIN) CB-839 HCl in patients with metastatic or unresectable MPNST is ongoing (https://clinicaltrials.gov/ct2/show/NCT03872427). Still, CB-839 resistance has been observed *in vitro,* whereby c-Myc induction takes place through epigenetic changes mediated by bromodomain-containing protein 4 (BRD4), which promotes transcription by recognizing acetylated lysines on histones. Indeed, CB-839 resistant cells are more sensitive to JQ1, a small molecule inhibitor of BRD4 [41]. Furthermore, glutamine dependency has been identified in lung adenocarcinomas where KRAS mutations coexist with Nf1 loss [42]. This work suggests that oncologic patient stratification for NF1 loss may uncover crucial targetable metabolic adaptations.

Similarly, the glutamine antagonist JHU395, a novel orally bioavailable prodrug designed to circulate in an inert form in plasma and to permeate and release the active drug within target tissues, is able to inhibit tumor growth in a murine flank MPNST model [43]. One of the major outcomes of JHU395 administration is the reduced usage of glutamine-dependent metabolites with a prominent effect on purine synthesis. Interestingly, glutamine utilization for anaplerotic purposes (*i.e.* supply of TCA intermediates such as glutamate, α-ketoglutarate and succinate) is not limited by JHU395. The different modes of action of drugs targeting glutamine metabolism indicate that multiple metabolic pathways in glutamine utilization might be critical for MPNST growth.

3.3 Lipid metabolism

During cancer growth, transformed cells experience nutrient and glucose shortage and must install metabolic adaptations to overcome these potentially harmful circumstances. Metabolic stress factors such as hypoxia and glucose deprivation increase expression of carnitine palmitoyltransferase 1C (CPT1C), member of a family of mitochondria-associated enzymes that regulate fatty acid metabolism. Its genetic ablation in a NF1 murine model delays tumor growth [44]. This finding exposes a susceptibility of NF1-related cancers to drugs targeting lipid metabolism when stressful conditions occur, as in the case of active chemotherapeutic regimens.

Lipid droplet accumulation has been reported in MPNSTs, which utilize both exogenous and endogenous lipids as a source of energy [45]. Indeed, either disruption of fatty acid oxidation and the use of the fatty acid synthase (FASN) inhibitors C75, orlistat and Irgasan reduce MPNST survival.

MPNSTs have been reported to secrete elevated levels of prostaglandin E2 (PGE2), an active lipid compound with hormone-like effects in animals [46]. It usually acts as an endocrine mediator of metabolic processes in homeostasis but also in inflammatory and neoplastic conditions. Remarkably, PGE2 receptor antagonists decreased the proliferation of MPNST cell lines. Prostaglandin administration has

also been linked to aberrant cAMP metabolism in MPNSTs that display two-fold increased cAMP levels compared to normal Schwann cells [47].

3.4 Connections between genetic mutations and metabolic changes

The HPGL/PCC syndrome, where loss-of-function mutations affect SDH and increase intracellular levels of the oncometabolite succinate, thus causing onset of pheochromocytoma and paraganglioma, is a proof-of-concept that metabolic changes can drive tumorigenesis. It is of note that NF1 patients can develop this kind of tumors in 5% of cases, whereas in non-NF1 related patients with HPGL/PCC history NF1 mutations have been reported in tumor cells [48]. This information, even though only correlative, is in accord with the observation that TRAP1 exerts a pro-neoplastic role in NF1 by inhibiting SDH, and suggests a possible overlapping path of metabolic adaptations existing between inactivation of NF1 and SDH components. Moreover, it must be highlighted that dysregulated signaling cascades can impinge on metabolic circuits, thus leading to neoplastic metabolic alterations either in the absence or in addition to specific mutations in metabolic enzymes.

Another interesting line of investigation links gene mutations to pro-neoplastic metabolic adaptations during neurofibroma growth. Indeed, it was reported that somatic mutations in mitochondrial DNA (mtDNA), which encodes 13 proteins of the OXPHOS machinery, are acquired and maintained by a high percentage of cutaneous and plexiform neurofibromas [49]. This suggests a possible positive selection in neoplastic cells for mutated mitochondrial genes, in keeping with observations that an aberrant mitochondrial respiration confers adaptive advantages to neurofibroma cells.

Take home message. Although the metabolic landscape of neurofibromin-deficient cells and MPNST has been only partially investigated, uncovering the adaptations in the metabolic circuits of these tumor cells may shed light on novel targetable actors. Furthermore, despite the genetic variability in MPNSTs characterized by different acquired mutations (e.g. TP53, p16, PRC, SUZ12, *CDKN2A,* etc.), there is the possibility of conserved derangements in metabolic pathways that may render MPNST vulnerable to selective targeting.

Perspectives. Beside cell autonomous changes in metabolism of neurofibromin-deficient tumor cells, their metabolic phenotype can be determined by alterations in intercellular communication within the tumor microenvironment. Recently it has been reported that fibroblast metabolic rewiring can promote growth of neural tumors [50]. Furthermore, beside the mitochondria to nucleus signaling mediated by succinate-dependent regulation of HIF1α and of epigenetic changes, this oncometabolite can exit tumor cells affecting immune cell responses. Thus, metabolic changes within neurofibromin null cells can affect the behavior of neighboring cells within the tumor microenvironment. Recent advances in immunotherapy approaches against MPNST growth have highlighted how these cancers might evade immune recognition and hijack immunological functions (e.g. tissue healing, angiogenesis, etc.) to their advantage. Whether the metabolic status of NF1-related tumors mediate the relationship between transformed cells and immune system is an exciting matter of investigation.

4. Conclusions

For a long time, pharmacological treatments suited for NF1-related neoplasms have been lacking. Only recently the first therapeutic approaches have been

translated from NF1 mouse models to patient bedside and further clinical trials are currently ongoing. Altogether, the major efforts in managing NF1-related neoplasms have been based on drugs targeting signaling transduction cascades such as RTK, RAS- RAF–MEK–ERK and PI3K-AKT–mTOR inhibitors. Selumetinib was the first drug approved in 2019 by the US FDA for pediatric NF1 patients with symptomatic and inoperable PN [51, 52] after a phase 2 clinical trial started a decade ago (https://clinicaltrials.gov/ct2/show/NCT01362803). Results indicate that 74% of patients display a partial response in terms of tumor volume shrinkage, and this is durable in 56% of patients. Albeit extremely positive, these results demand the urgent development of additional treatments. Previous attempts of targeting signaling cascades in neurofibroma microenvironment through imatinib mesylate administration, a dual SCF/cKIT inhibitor, have shown modest response rates limited only to small tumors [53] (https://clinicaltrials.gov/ct2/show/NCT01673009). Cabozantinib, an inhibitor of multiple tyrosine kinases among which c-Kit, vascular endothelial growth factor (VEGF) receptor (VEGFR)2, MET, RET, FMS-related RTK 3 (FLT3) and TAM family receptors (tyrosine kinases AXL, TYRO3 and MERTK) is now under study in a phase II trial against progressive or symptomatic, inoperable PN (https://clinicaltrials.gov/ct2/show/NCT02101736) as it has shown promising results in Nf1-mutant mice [54].

As for glioma, chemotherapy remains the first line treatment. More recently, epigenetic-based approaches in fighting MPNST growth have emerged [55] and drugs targeting the immune checkpoints are considered the emerging therapeutic option with ongoing clinical trials [56, 57] (https://clinicaltrials.gov/ct2/show/NCT02691026).

In this scenario, beside the recently reviewed pharmacological options for MPNST treatment [58–60], targeting the metabolic features of NF1-related tumors constitutes an additional, promising therapeutic option. Although multiple metabolic routes have been shown to be affected in NF1 tumorigenesis, metabolic based anti-neoplastic approaches are limited in the field (BeGIN clinical trial) and others are at the preclinical stage (**Figure 3**). A recent report has resumed the idea of targeting the glycolytic pathway [61]; however, the drug employed, *i.e*. 3-bromopyruvate, has already been dismissed from past clinical trials for excessive and life-threatening toxicity.

As for MPNST, complete surgical excision with clear margins remains the only treatment in the case of a localized cancer. Given the lack of efficacy in targeting unique aspects of MPNST disease biology, some benefits could hopefully come from combinatorial therapeutic designs that consider and include innovative rational therapies, such as targeting bioenergetic circuities.

In this direction, despite the genetically heterogeneous phenotype of NF1-related malignancies, the annotation of conserved metabolic adaptations in the progression towards MPNST might open space for innovative therapeutic interventions [62].

Perspectives. Advancements in animal modeling of NF1-related neoplasms are meant to refine the understanding of PN tumorigenesis and put the basis for testing multi-targeted drug therapies and adaptive tumor response. For instance, atypical neurofibromas with an uncertain transforming potential have been recapitulated by *Cdkn2a* loss [63]. Uncovering the potential metabolic adaptations of the transitional stages of NF1 tumors from the benign to the malignant ones may equip clinicians with metabolic biomarkers to be monitored during NF1 patient surveillance.

Furthermore, the understanding of the metabolic interplay between cancer cells that have lost neurofibromin and other cell types present in the tumor microenvironment might uncover metabolic susceptibility of these cancers. For instance, MPNSTs display an increased number of macrophages with respect to PNs and

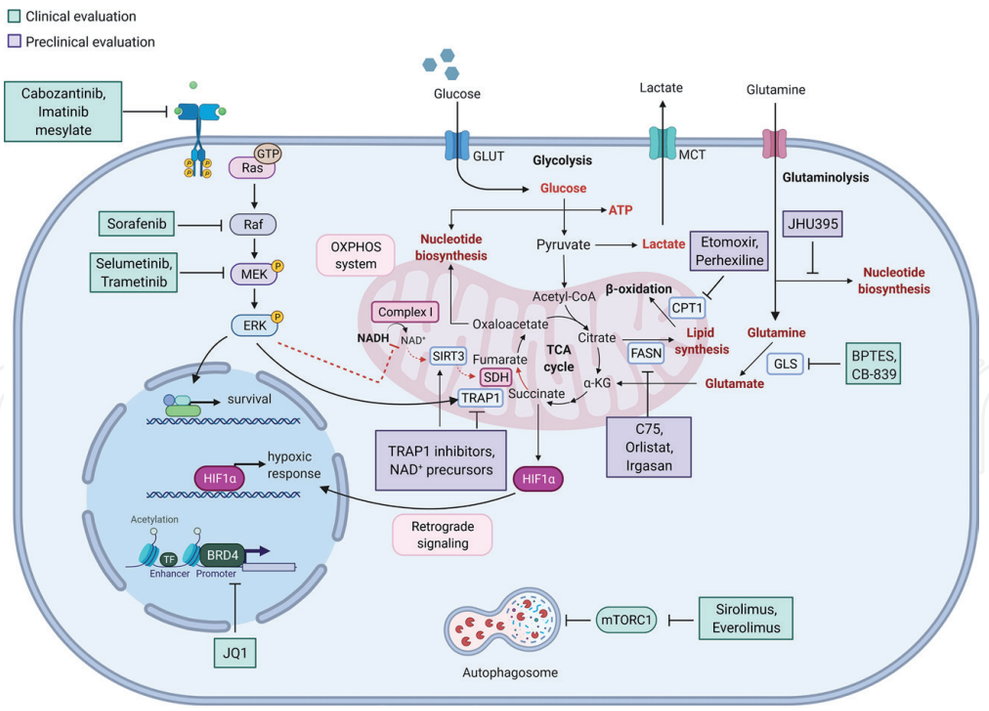

Figure 3.
Treatment options against NF1-related neoplasms. Drugs under clinical (green) and preclinical (violet) evaluation against NF1-related tumors are reported.

are highly glutamine-addicted. It is known that macrophages sense the lack of glutamine and install a synthetic pathway for glutamine supply based on glutamine synthetase induction. This metabolic rewiring characterizes the pro-tumorigenic polarization towards a tumor-associated macrophage phenotype. Given these tight and crucial metabolic crosstalks between tumor cells and the immunologic compartment, it can be envisioned that targeted therapies are accompanied by metabolic-based treatments hitting both neoplastic and environmental cells in order to overcome potential cancer resistance (e.g. CB-839 and JQ1, which combines metabolic and epigenetic treatments).

PET scans with labeled glucose uptake evaluation can provide an extremely useful tool for monitoring lesions at high potential for growth and at risk for malignant transformation; regular imaging is suggested especially in symptomatic neurofibromas [64]. We expect that metabolic tracking of additional nutrients such as glutamine could be employed in NF1 patients for the unraveling of metabolically active lesions. Imaging of labeled glutamine is currently under evaluations in cancer patients and has the potential of predicting cancer response to metabolic targeted therapies, thus helping the guidance of therapeutic decision-making [65, 66].

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Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

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