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The Warburg effect in Multiple Myeloma and its microenvironment

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Abstract

This review highlights the current state of knowledge about the metabolism of cancer cells, especially with respect to "Warburg effect" in the pathogenesis of Multiple Myeloma (MM). Various pathways are known to contribute to the Warburg effect, characterized by an increased anaerobic glycolysis rather than mitochondrial oxidative phosphorylation (OXPHOS), resulting in elevated levels of lactic acid even in the presence of sufficient oxygen. For one thing it was shown, activation of the PI3K/Akt/mTOR leads to enhanced expression of nutrient transporters and stimulation of glycolysis. In particular, glucose transporter (GLUT) 1, 4, 8 as well as 11 show elevated expression in MM and therefore enhanced glycolytic flux as well. Another important route manifesting the Warburg effect is peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and its dual role within the production of reactive oxygen species (ROS). Furthermore, the stabilization and transcription of hypoxia-inducible factor 1 α (HIF1α) under tumor-attributed hypoxic conditions shows promising downstream mechanisms such as histone deacetylase (HDAC), which can be targeted and serve as adjuvant therapy to prolong overall survival of MM patients. In this paper we review the most promising and researched targets of the Warburg signaling are represented and analyzed upon their suitability as a therapeutic target in MM.

1. Introduction

1.1 Multiple Myeloma

Multiple Myeloma (MM) is one of the most prevalent hematological cancers with about 86.000 new cases per year globally [1]. MM is a hematological plasma cell malignancy which is characterized by indolent, isotype-altered plasma cells (PCs), located within the bone marrow (BM) and shows a profound genetic and phenotypical heterogeneity. Malignant PCs accumulate in the BM where homeostatic interactions between Multiple Myeloma cells (MMCs) and the BM microenvironment are aberrant and shifted towards survival and growth of tumor cells, angiogenesis, bone destruction, drug resistance and immunodeficiency [2, 3]. Expanding of infiltrated malign PCs, replacement of normal BM cells and therefore the increase of antibodies defines the MM subtype and triggers the most common symptoms: hypercalcemia, renal failure, anemia and bone lesions, also defined by the CRAB-criteria [4].

Despite efficient treatments regimes of high-dose chemotherapy followed by a combination of immunomodulatory drugs (IMiDs), proteasome inhibitors or monoclonal antibodies, MM still remains an incurable disease with poor prognosis. However, novel treatment regimes radically improved response and overall survival rates [5].

1.2 Interaction between Bone marrow mesenchymal stromal cells and MMC

Bone marrow mesenchymal stromal cells (BMMSCs) represent a crucial component in creating a MM supportive microenvironment. MM derived BMMSCs alter proliferation, anti-apoptotic signaling, drug-resistance, migration and invasion by either direct cell-cell contact or via secretion of cytokines and chemokines [6, 12]. It is well known, that MMCs link to adjacent BMMSCs via its integrins (namely VLA-4, LFA-1, MUC-1) and stromal cell adhesion molecules (VCAM-1, ICAM-1) as shown in figure 1.

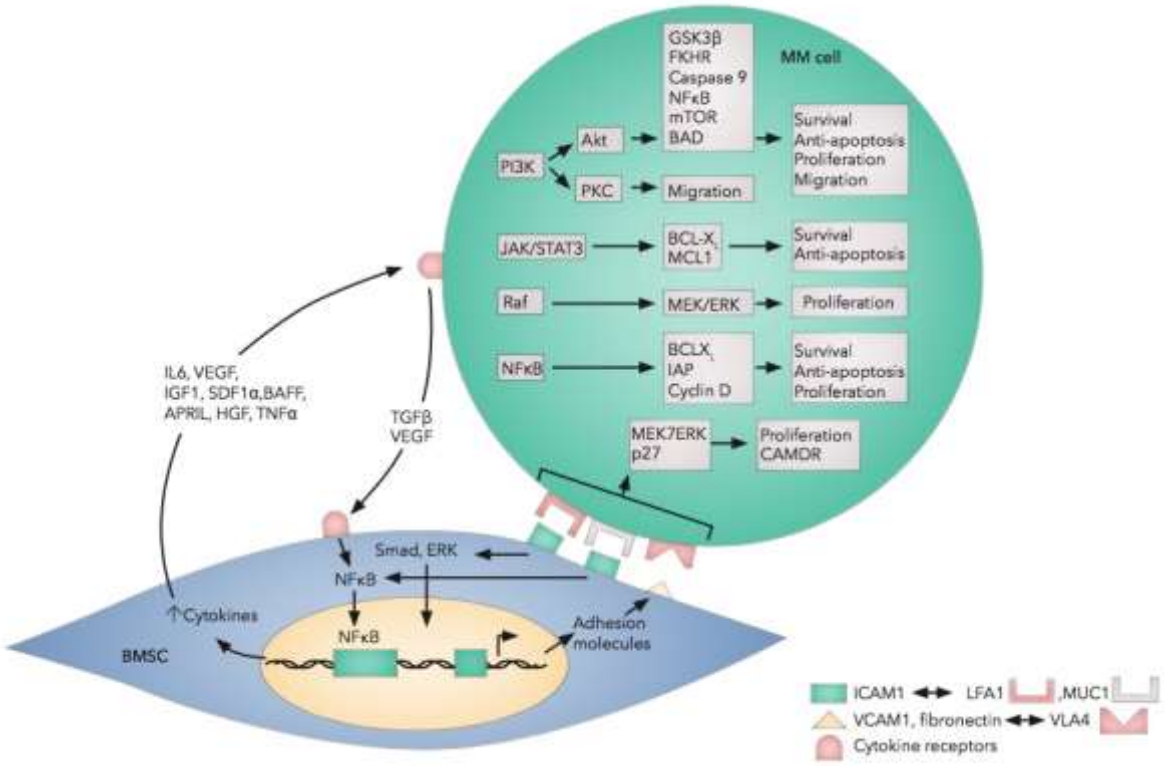


Figure 1: Multiple Myeloma cells (MMC) and their interaction with the microenvironment. The adhesion of MMCs and bone marrow mesenchymal stromal cells (BMMSCs) initiate cytokine-mediated proliferation, survival and migration. Binding of MMCs and BMMSCs via intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1), lymphocyte function-associated antigen 1(LFA1), mucin 1 cell surface associated (MUC1) and very late antigen-4 (VLA4) results in cytokine-upregulation by both cell types. Cited cytokines activate major signaling cascades: (i) the phosphatidylinositol 3-kinase (PI3K) - protein kinase B (Akt) - mechanistic target of rapamycin (mTOR) pathway; (ii) the janus kinase (JAK) - signal transducer and activator of transcription 3 (STAT3) axis; (iii) the Ras–Raf–MAPK kinase (MEK) - extracellular signal-regulated kinase (ERK) pathway and/or (iiii) nuclear factor κB (NFκB), which combined evoke cancer cell survival through impaired apoptosis, migration, proliferation and cell-adhesion-mediated drug resistance (CAMDR). This figure is modified from: Teru Hideshima, Constantine Mitsiades, Giovanni Tonon, Paul G. Richardson & Kenneth C. Anderson. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets *Nature Reviews Cancer* 7, 585-598 (August 2007)|doi:10.1038/nrc2189.

The interaction results in activation of NFκB-pathway, subsequently initiating cytokine release (IL-6, VEGF, IGF-1 a.o.), which in turn intensifies the interaction between BMMSCs and MMCs. MMCs secrete inflammatory molecules such as TFG-β and VEGF, analogously activating NFκB tumorigenic signaling [7]. The enhanced activation of NFκB and the associated overexpression of adhesion molecules, may provoke therapy resistance [8]. Based this context, integrin-mediated apoptosis-resistance is called "cell-adhesion mediated drug resistance" [9]. Furthermore, IL-6 and VEGF depict a pivotal role in the aberration of multiple signaling pathways such as PI3K, JAK/STAT3, Raf and NFκB [7].

Regarding metabolic effects, the interaction appears to facilitate MMC survival and proliferation, for instance via reduction of reactive oxygen species

(ROS) and thus creating a defense-mechanism against ROS-induced apoptosis [10]. Considering stress-related molecules, reduced mitochondrial stress response was observed in BMMSCs of MM patients compared to healthy donors, manifested by a decreased expression of SIRT3 (NAD-dependent deacetylase sirtuin-3, mitochondrial). SIRT3 represents a major regulator of the mitochondrial function, hence reduced expression could account for accumulation of ROS in BMMSCs cultured *in vitro*. Intriguingly, exposition of BMMSCs to MMCs in co-culture experiments induced an increase of SIRT3 levels, mitochondrial stress response was enabled and ROS levels decreased [11]. Moreover, the interaction upregulates expression of monocarboxylate transporters (MCT1 and MCT4) in MMC, which might be responsible for lactate-discharge. In turn, enhanced lactate levels stimulated MMC proliferation even further and inhibition of this interaction via MCT-inhibition with α-Cyano-4-hydroxycinnamic acid (CHCA) resulted in MMC-apoptosis [12]. Consistent with data from Walters et al., an increased expression of CD147 - a transmembrane glycoprotein considered responsible for MCT1 and 4 expression - in both cell types was observed [13].

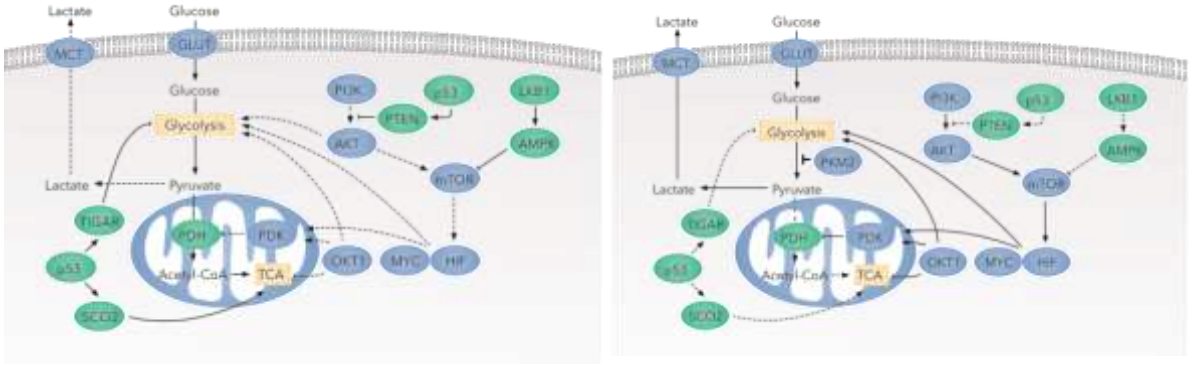
1.3 The Warburg effect

Bioenergetic and biosynthetic dependencies of cancer cells more and more enter the limelight as potential therapeutic targets [14]. Cancer cell survival and proliferation depend on metabolic processes like glucose-uptake via altered glycolysis, also known as "Warburg effect". Rather than mitochondrial oxidative phosphorylation (OXPHOS), cancer cells show increased anaerobic glycolysis within the cytosol, producing high levels of lactic acid [15]. This metabolic reprogramming of cancer cells into anaerobic glycolysis characterizes the adaptive properties of carcinogenesis as a response to the hypoxic tumor microenvironment. Even in the presence of oxygen, cancer cells favor fermentation over mitochondrial OXPHOS [16]. In detail, mitochondrial OXPHOS consists of three steps: first, pyruvate generated by cytoplasmic glycolysis is decarboxylated and converted to Acetyl coenzyme A (acetyl-CoA), which subsequently enters the tricarboxylic acid (TCA) cycle within the mitochondrial matrix. Oxidative phosphorylation as the last step occurs in the inner mitochondrial membrane and provides energy by building up an electron transport chain via the formation of a proton gradient. In total, cancer cells have to compensate for an 18-fold ATP production by choosing the approach of aerobic glycolysis followed by lactic acid fermentation instead of OXPHOS [17]. Why do proliferating cells still choose such a wasteful path of metabolism? On the one hand, cancer cells can utilize the most surplus extracellular nutrient glucose to generate ATP. Despite the low energy yield, ATP generated by glycolysis can outweigh OXPHOS if glucose flux is high enough. On the other hand, glucose degradation provides important intermediates for cell growth: (i) by phosphorylating glucose to glucose 6-phosphate through hexokinase (HK), the intermediate is channeled to the pentose phosphate pathway (PPP). The ensuing formation of ribulose 5-phosphate serves as a precursor for the synthesis of nucleotides, NADPH as a reducing equivalent is essential for several reductive biosynthesis such as fatty acid synthesis [17]. (ii) in the last step of glycolysis, pyruvate is formed by means of pyruvate kinase (PK) from phosphoenolpyruvate. The ATP providing isozyme PK-M2 is discussed to function as rate limiting for pyruvate generation as it is mainly expressed in its dimeric form with low affinity to its substrate. Hence, all glycolytic intermediates preceding the introduction of PK accumulate and are used for building up profitable metabolites

for tumor cells, e.g. nucleic acids, fatty acids or amino acids [17, 18]. (iii) another capacity of PK-M2 overexpression is its influence in mitochondrial fission and fusion by affecting the mitochondrial fission protein dynamin-related protein 1 (Drp1) and therefore the mitochondrial distribution and morphology. The mitochondrial quantity as well as the mitochondrial ATP yield declines in coherence with mitochondrial fusion prevailing mitochondrial fission. Furthermore, increased PK-M2 expression indicates decreased p53 protein levels and shortened p53 half-life period [18].

2 Metabolic influences on tumor progression

To highlight the significance of the Warburg effect in Multiple Myeloma pathogenesis, numerous pathways and key transporters supporting the disease progression will be discussed.



2a Quiescent normal cell

2b Proliferating tumor cell

Figure 2a-b: Signaling pathways driving the Warburg effect. The shift to aerobic glycolysis in proliferating tumor cells (2b) from quiescent normal cells (2a) is promoted by various oncogenic signaling cascades. (a) PI3K activates AKT, which for one triggers glycolysis and on the other hand mTOR. Among other metabolic effects, mTOR affects glycolytic characteristics by elevating HIF levels leading to PDK increase and subsequent inhibition of pyruvate entry into the TCA cycle. In combination with HIF, MYC activates multiple genes that encode glycolytic proteins and also raises mitochondrial metabolism. (b) Tumor suppressor p53 counteracts the glycolytic phenotype by alleviating anti-apoptotic properties of PTEN, affecting apoptosis regulator TIGAR and increasing mitochondrial metabolism via SCO2. (c) OCT1 reacts in an adverse manner by activating transcription of glycolytic genes and repressing OXPHOS associated genes. (d) Primary assembly of pyruvate kinase isoform M2 decelerates pyruvate kinase reaction and redirects substrates to alternative degradation routes rather than direct OXPHOS. This figure is modified from: Rob A. Cairns, Isaac S. Harris, Tak W. Mak. Regulation of cancer cell metabolism. Nature Reviews Cancer 11, 85-95 (February 2011) | doi:10.1038/nrc2981

2.1 PI3K/Akt/mTOR signaling

Phosphatidylinositol 3-kinase (PI3K) Class I is a lipid kinase and a pivotal regulator in metabolic processes by converting the second messenger phosphatidylinositol (4,5)-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP3), which triggers protein kinase B (Akt) and other downstream signaling pathways. PI3K consists of four homologous isoforms - PI3K α, β, γ and δ - which are activated by either tyrosine kinases or, in the case of isoform γ, by G protein–coupled receptors (GPCRs) in

response to growth factors [19]. In normal cells, PI3K is strictly controlled by dephosphorylation of PIP3 through phosphatase and tensin homolog (PTEN), a protein which operates as a tumor suppressor and therefore as antagonist for PI3K activity. Proliferating cells show abrogated PTEN effect mechanism leading to (i) an increased expression of nutrient transporters facilitating increased glucose uptake; (ii) stimulation of glycolysis due to of HK and phosphofructokinase via Akt signaling; (iii) heightened transcription of lipogenesis as well as glycolysis related genes; (iiii) Akt-mediated mechanistic target of rapamycin **(**mTOR) activation and thus elevated protein expression [17, 20]. On the one hand, PTEN serves as antagonist against PI3K downstream activation and at the same time suppresses osteoclast differentiation resulting in MM characteristical bone lesions. MMCs invading into the bone marrow secrete osteoclast-stimulating factors, which in turn stimulate stromal cells and osteoblasts to secrete receptor activator of nuclear factor kappa-B ligand (RANKL) - a ligand considerably increased in MM. PTEN is described to negatively regulate the RANKL-activated Akt survival signaling pathway and the cell migration in osteoclast precursors [21]. mTOR is a serine/threonine protein kinase and the catalytic component of the protein complex mTOR Complex 1 (mTORC1) together with its binding partners (a) regulatory-associated protein of mTOR (Raptor), (b) mammalian lethal with SEC13 protein 8 (mLST8 or GβL), (c) proline- rich AKT substrate 40 kDa PRAS40 and (d) DEP-domain-containing mTOR-interacting protein (DEPTOR) [22]. mTOR is also a part of mTORC2, but instead of raptor and PRAS40, it contains the proteins rictor, mSin1 and protor. The two multiprotein complexes differ in its function: mTORC1 is a key regulator of protein synthesis by phosphorylating S6 Kinase 1 (S6K1) and the eukaryotic translation initiation factor 4E-binding protein 1(4E-BP1), whereas mTORC2 phosphorylates its downstream effectors Akt and Serum/Glucocorticoid regulated kinase 1 (SGK1), thus controlling cell survival. Binding partner DEPTOR, which is present in both multiprotein complexes, normally functions as an inhibitor to mTORC1 and mTORC2 pathways and their downstream mechanisms. In Multiple Myeloma, a consistent overexpression inhibits mTORC1 which subsequently activates the PI3K-mTORC2-Akt signaling cascade resulting in apoptosis resistance and correlates with poor survival [17, 22]. Further on, the extracellular matrix protein reelin is discussed to drive the PI3K-Akt-mTOR pathway leading to increased cell proliferation and glycolysis by elevated lactate dehydrogenase (LDH) and pyruvate dehydrogenase kinase 1 (PDK1) levels. On a more nuclear approach, Reelin also intervenes in the JAK-STAT3 axis by triggering transcription factor STAT3, which results in suppressed apoptosis via bcl-xl and proliferation via cyclin D1 activation [23, 24].

2.2 Glucose transporters as metabolic targets in Multiple Myeloma progression

In order to compensate elevated energy consumption in tumor cells, increased expression of nutrient transporters on the plasma membranes is one approach to respond to the increased metabolic rate. In MM, high glucose flux is facilitated by upregulation of glucose transporters GLUT1, GLUT4, GLUT8 as well as GLUT11. Several studies show, that therapeutic inhibition of glucose metabolism might be an appropriate strategy to treat MM but it has to be further explored *in vitro*, *in vivo* and in clinical studies as e.g. the inhibition of hexokinase by 2-deoxyglucase and lonidamine failed in clinical trials [25]. GLUT1 frequently elevated in various types of cancers. Like other GLUTs, it normally resides in small vesicles storing the protein in their membrane which

translocate the protein through exocytosis to the plasma membrane. An explicit correlation between the PI3K-Akt pathway and the translocation of glucose transporters could be determined in case of GLUT1 and 4 [26]. Research suggests GLUT4 dependent glucose and lactate production and in the case of GLUT8 knockdown, cell death and growth inhibition and modest inhibition of glucose transport and lactate production is documented. Minor effects concerning glucose uptake and lactate extrusion are depicted in GLUT1 and GLUT11 as cell lines undergo growth arrest if exposed to glucose deprivation [25]. Intervening the Warburg effect via GLUT4 inhibition is possible by applying the HIV protease inhibitor ritonavir, which has an off-target effect on the glucose transporter. The agent evokes reduced myeloma proliferation and viability and simultaneous increased chemosensitivity *in vitro* [25]. In recent clinical trials, concurrent administration of ritonavir and metformin to diabetic HIV patients has been well tolerated, which makes the regimen a potential application for MM patients. *In vitro* research applying the method has been examined *in vivo* for chronic lymphocytic leukemia (CLL) and provides promising results as metformin additionally inhibits mitochondrial complex I [27].

Moreover, enhanced glucose turnover is a critical driver of promoting proliferation and evading of apoptosis by influencing of B-cell lymphoma 2 (BCL-2) proteins such as myeloid cell leukemic factor 1 (MCL-1). Glucose-deprivation of MMCs results in decreased MCL-1 expression but did not obligatory induce apoptosis, which makes the MCL-1-GLUT4-mTOR axis a potential point of interference, which is currently being inspected for profitable effects and prolonged life span by administering the cyclin-dependent kinase (CDK) inhibitor seliciclib [25, 28, 29].

2.3 The dual role of PGC-1α and ROS effects in Multiple Myeloma

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) as a transcriptional co-activator regulates multiple genes involved in energy metabolism, in particular the mitochondrial biogenesis [30]. It influences GLUT4, which is as already stated upregulated in MM, the maintenance of Mcl-1 expression, regulates nuclear respiratory factors (NRFs) and also the binding of estrogen-related receptor α (ERR-α), leading to enhanced angiogenesis and expression of vascular endothelial growth factor (VEGF). *In vitro* experiments determine elevated PGC-1α levels and also upregulation in metabolic downstream factors. A strong reliance between PGC-1α and VEGF as well as suppressed angiogenesis when treated with siPGC-1α is ascertained. Inhibition of PGC-1α evokes decreased GLUT4 expression *in vitro* and could therefore lead to reduced lactate production in its downstream as in accordance with GLUT4 findings, proliferation rates also diminished by targeting cell lines with siPGC-1α. Endorsing antiangiogenic effects of drugs applied in practice (e.g. lenalidomide) with PGC-1α-inhibitors might hence improve effects in clinical use [30]. Apart from the significance in glycolytic pathways, PGC-1α also resembles a complex actor in ROS production and apoptosis. ROS levels in hypoxic cancer cells are reported to induce PGC-1α expression as well as subsequent detoxification via superoxide dismutase 2 (SOD2), catalase and glutathione peroxidase [31]. Research shows, that cells treated with bortezomib showed higher levels of PGC-1α, accompanied with increased expression of SOD-2 and catalase than in those without treatment. One can hypothesize, that PGC-1α promotes MMC survival in conditions of chemotherapy as inhibition of PGC-1α in MMCs showed decreased expression levels of SOD-2 and catalase along with elevated ROS expression and increased toxicity of chemotherapy drugs. It seems

contradictory, that PGC-1α stimulates mitochondrial biogenesis, thus generating more ROS and leading to more effective chemotherapy, when PGC-1α suppresses ROS accumulation at the same time and results in the dysfunctional effect of chemotherapy. This phenomenon might be explained by the bypass of OXPHOS via anaerobic glycolysis within the Warburg effect, which evades high ROS production. Summarizing, the suppression of PGC-1α by siRNA increases the levels of ROS and thereby enhances toxicity of bortezomib *in vitro*, which makes PGC-1α a potential target to improve the antitumor effect of bortezomib [32]. Complementary to the subject of oxidative stress and proteasome inhibitors, combinational treatment of MMCs with bortezomib and other ROS-inducing agents could already point out promising therapy-strategies: for one, inhibition of the oncoprotein mucin 1 C-terminal subunit (MUC1-C) synergized with bortezomib administration led to higher ROS-levels and re-sensitization of bortezomib resistant MMC. Besides MUC1-C, co-treatment with (i) next-generation analog of bortezomib carfilzomib, (ii) recently approved histone deacetylase (HDAC) inhibitor panobinostat, resulted in ROS-dependent apoptosis [33].

2.4 Metabolic adaptations arising from HIF1 transcription

Reduced oxygen availability -hypoxia- evokes 2 main phenotypic ramifications in proliferating cells: increased glucose consumption and lactate production due to the shift in energy production via anaerobic glycolysis. This shift is accompanied by HIF1α expression, which is activated in hypoxic stress. Under normoxic conditions, HIF1α accumulation is inhibited by prolyl hydroxylation and ubiquitination on the part of the von Hippel–Lindau (VHL) tumor suppressor followed by proteasomal degradation. In a hypoxic state, prolyl hydroxylation is decreased and thus HIF1α is stabilized and transcription achieved via the HIFβ subunit (ARNT) as shown in figure 3 [17].

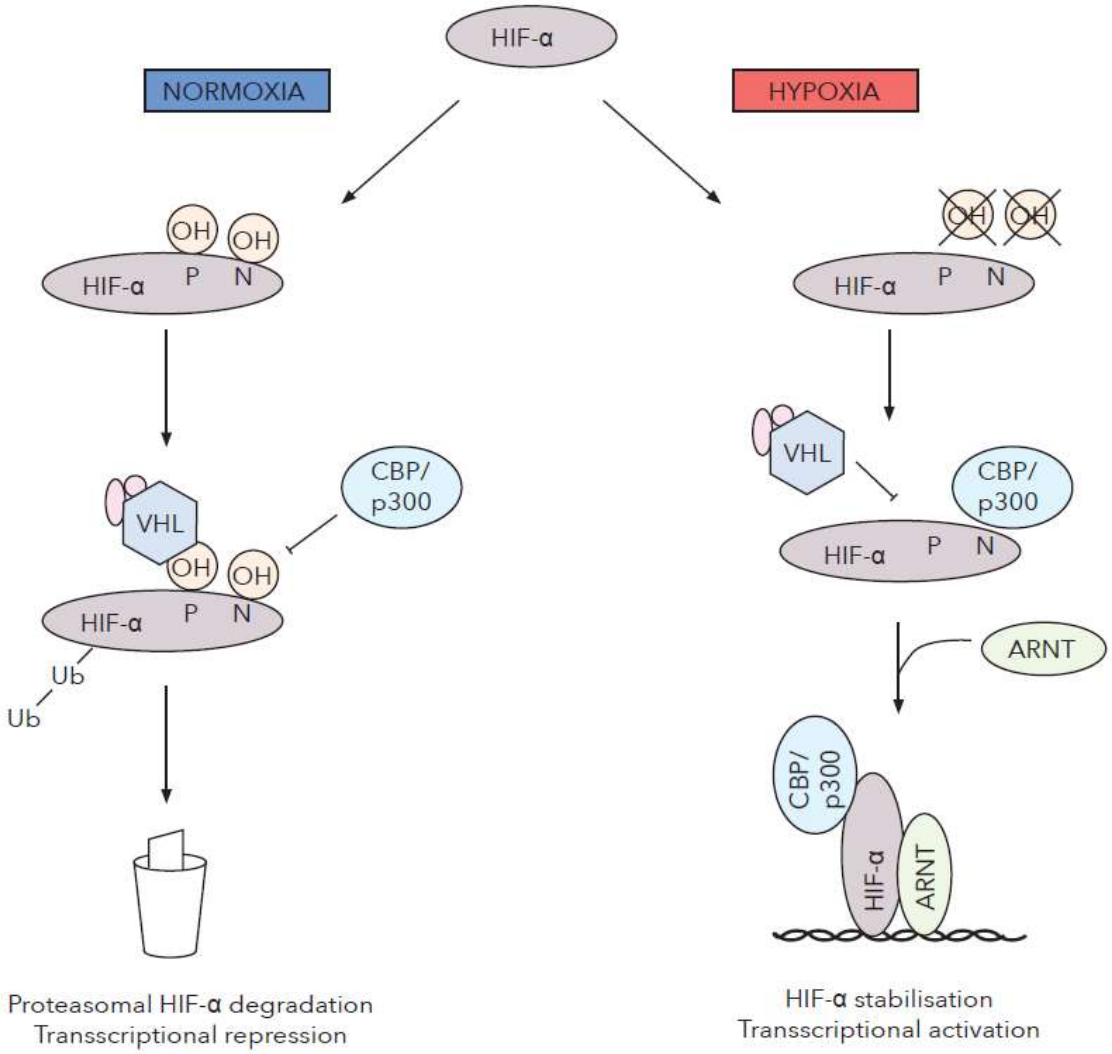


Figure 3: Regulation of the hypoxia-inducible transcription factor (HIF) complex depending on the oxygen status. Under normoxic conditions, oxygen-dependent enzymes inhibit the assembly of HIF complex by hydroxylation of asparagine and proline residues in the HIF-α subunits. These alterations inhibit coactivator binding and promote von Hippel–Lindau (VHL) recruitment, hence proteasomal degradation. Hypoxic conditions prevent enzyme activity, leading to the assembly of stable, transcriptionally active HIF. This figure is modified from: Martin SK, Diamond P, Gronthos S, Peet DJ, Zannettino AC. The emerging role of hypoxia, HIF-1 and HIF-2 in multiple myeloma. Leukemia 2011; 25: 1533–42.

HIF1α has various downstream mechanisms concerning the Warburg effect: for one, it triggers the upregulation in GLUT genes, glycolytic enzymes, and LDH. Additionally, it assists upregulation of PDK1 -the negative regulator of PDH- which leads to a diminished flux via OXPHOS in favor of anaerobic glycolysis and therefore reduced mitochondrial ROS production. Interestingly, the above discussed PI3K signaling cascade is reported to also regulate HIF1α on a transcriptional basis. Thereby, in malignancies such as MM where HIF1α expression is sustained by a dual mechanism, precisely hypoxia and activated PI3K signaling, targeting HIF1α might give rise to efficient therapy strategies [17, 34, 35]. Specific agents such as ROS, growth factors, cytokines and the activated PI3K-Akt-mTOR pathway can activate HIF1α even under normoxic

conditions [36]. Recent research investigating the close interaction between MMCs and their microenvironment regarding HIF1α discovered, that co-cultivation of MMCs and BMMSCs stimulates the production of growth factors and anti-apoptotic factors, such as interleukin 6 (IL-6), insulin-like growth factor 1 (IGF-1), stroll cell derived factor 1α (SDF-1α) and vascular endothelial growth factor (VEGF). In contrast to most organs, the BM microenvironment represents a hypoxic compartment which stabilizes the active form of HIF1α. Experiments inhibiting HIF1α by a 3rd generation antisense oligonucleotide called EZN-2968 showed decreased HIF1α expression when MMC cell lines were co-cultured with primary BMMSCs. Additionally, viable cells diminished significantly performing a MTT cytotoxicity assay when co-cultivated and supplemented with IL-6. Further effects of co-cultivation under HIF1α inhibition were decreased cytokine secretion by MMCs: IL-10, IL-6, TNFα, VEGF and INFγ exhibited significant down-regulation. Adhesion-related examinations showed reduced adherence under EZN-2968 treatment, possibly resulting from the secretion of growth factors and the thereby enhanced adhesion of MMCs to the bone marrow. Co-cultivation as well as IL-6 treatment can also activate the mitogen-activated protein kinase (MAPK) pathway, as protein expression of Erk 1/2 as well as Akt increased, facilitating MMC proliferation. These findings verify the assumption, that the close interaction between BMMSCs and its adjacent MMCs increases tumor proliferation and crosstalk between notable metabolic signaling pathways [35]. Although co-culture findings lack in *in vivo* and clinical data to attest anti-proliferative and anti-angiogenic effects HIFα, in-vitro settings provide optimistic premonitions for potential utility as therapeutic target. What has already been investigated is the use of panobinostat - a HDAC inhibitor - which shows effective anti-MM activity *in vitro* as well as *in vivo*. Panobinostat does not target HIFα degradation per se, but it reduces its binding capacity and thus its transcriptional activity. In relapsed/refractory MM patients, the HDAC inhibitor could already successfully overcome the resistance to bortezomib and lenalidomide by targeting resistance to their initial antiangiogenic impact [36].

2.5 Autophagy as a tumor suppressor in Multiple Myeloma

Autophagy represents a crucial metabolic stress response and provides an important physiological role as cellular waste disposal system. When faced with metabolic alterations such as the Warburg effect, tumor specific pathways are activated and adversarial counter the attributed effects of autophagy. Pathways such as PI3K-Akt-mTOR impact autophagy in an inhibitory-manner, whereas tumor suppressors as PTEN or HIF1α assist autophagy. Two important key mediators in autophagy are the transcription factor p53 and the AMP-activated protein kinase (AMPK) [17]. p53 attributed features include activation of genes inducing cell cycle arrest, senescence and apoptosis in case of hypoxia, oxidative stress or DNA damage. It also indicates involvement in metabolic control driven by AMPK-phosphorylation and mTOR activation. In metabolic stress, p53 is able to intensify AMPK-dependent effects through multiple feedback loops that again activate AMPK-signaling. Activation of the AMPK-p53 axis initiates catabolic metabolism, e.g. enhanced β-oxidation of fatty acids through enzyme-stimulation of carnitine palmitoyltransferase (Cpt1) which regulates mitochondrial fatty acid import. Contributing to the metabolic effects by fatty acid is the tumor cell typical upregulation of fatty acid synthase (FASN) supplying more energy resources and supporting cancer cell survival and proliferation [17, 37]. Inhibition of Cpt1 by

etomoxir - a specific inhibitor of Cpt 1, which is applied in patients with chronic heart failure - or FASN by orlistat - a FDA-approved anti-obesity drug that specifically targets FASN- reduced cell viability and proliferation in MMCs *in vitro* [37]. Apart from p53 interplay with AMPK and the fatty acid synthesis, it can also influence glycolysis and OXPHOS through transcriptional regulation of fructose-2,6-bisphosphatase TP53-induced glycolysis regulator (TIGAR) as well as by production of cytochrome c oxidase (SCO2). TIGAR expression lowers fructose-2,6-bisphosphate levels, resulting in an inhibition of glycolysis and an overall decrease in intracellular reactive oxygen species (ROS) levels. At the same time, loss of p53 can compromise the mitochondrial respiratory chain and support a switch from OXPHOS to glycolysis by SCO2 regulation [17]. By protecting cells from excessive ROS, TIGAR may mediate some of the tumor suppressor activity of p53 but could also contribute to tumorigenesis. Thus, the role of p53 in autophagy remains controversial and seems to modulate apoptosis in a cell-type-dependent manner.
Secondly, AMPK is also a key mediator in autophagy. The kinase responds to the ratio of AMP:ATP and is activated under metabolic stress, where it promotes ATP production, increases catabolic metabolism and conserves ATP by inhibiting anabolic pathways. AMPK has several downstream mechanisms including increased glycolysis, lipolysis, fatty acid oxidation, inhibition of lipogenesis, cholesterol formation, as well as protein synthesis. Analogous to p53 findings, AMPK effects are controversial, supposedly acting as either a tumor promoting or a tumor suppressing depending on the context [38]. On the one hand, AMPK marks anti-Warburg effects by showing tumor suppressing features such as down-regulation of HIF1α, inhibition of mTORC1 activity and *de novo* lipogenesis. Contrary it is also described that AMPK may induce autophagy by (i) phosphorylation of the unc-51-like kinases (ULK), (ii) promoting fatty acid oxidation to generate ATP, (iii) change transcriptional factors by phosphorylating core histone H2B, (iv) upregulating intracellular NADPH levels through increased fatty acid oxidation and the inhibition of fatty acid synthesis in order to neutralize cytotoxic ROS. Also, under glucose deficiency, both AMPK and Akt are activated and coordinately support cell survival. Regarding MM, *in vitro* studies show conflicting results. For one, AMPK activation by 5-aminoimidazole carboxamide riboside (AICAR) inhibited cell growth in myeloma cell lines whereas AMPK inhibition by BML-275 (Compound C) induced apoptosis in the same cells and therefore provides the contrary effect [39, 40]. In order to obtain unambiguous results for AMPK effects in autophagy and MM in general, more research has to go into the cross-talk mechanisms auf the kinase and other pathways.

3 Conclusion

Having investigated the most relevant pathways attributing to the Warburg effect it becomes clear, that MMCs alone do not account for the progression of MM since the interaction with its microenvironment is a driving force in tumor proliferation. Even though novel therapeutic measures already brought tremendous advances in life expectancy compared with only a few years ago, one should not neglect metabolic processes such as the Warburg effect when treating MM with the latest developed drugs, e.g. monoclonal antibody therapy.
The most simple and obvious example, that metabolism is essential in carcinogenesis and progression of not only MM is the role of the body mass index (BMI) within the disease progression. On the one hand, obesity (a BMI over 25) lowers adiponectin levels, a protein hormone secreted by adipose tissue

pioneering in metabolic regulation as it decreases proliferation *in vitro* by influencing glycolysis, fatty acid oxidation, insulin sensitivity among others, and increases the expression of both cytokines as well as growth factors [41]. Additionally, first cohort studies demonstrate the transformation of the 'preliminary stage' monoclonal gammopathy of undetermined significance MGUS to MM on account of obesity [42]. On the contrary, obesity can also promote survival after MM diagnosis. In several cohort studies, obese patients benefit in contrast to normal weight or underweight patients as they store more energy reserves and physical strength in times of often observed disease-related weight loss in the months leading up to MM diagnosis [41].

Within this review, the focus was put on the glycolytic side of the Warburg effect, nevertheless the fatty acid metabolism should not be neglected as potential metabolic target, as fatty acid synthase has low expression levels in normal tissues but is upregulated in cancer cells. Combing the standard treatment regimen with agents targeting metabolic pathways as adjuvant therapy appears to be a promising strategy according to the stated *in vitro*, *in vivo* as well as first clinical trial results. A few of the currently investigated agents such as ritonavir, metformin or panobinostat already represent inhibiting effects on tumor progression, making other targets within the Warburg effect interesting research fields as well. Most recent trials targeting mTOR via rapamycin or HDAC via entinostat hint towards diminished tumor growth, making the PI3K an even more interesting pathway to focus on, when it comes to metabolic management within MM [43]. Interestingly, decreased expression of the mTORC subunit DEPTOR in MM cell lines caused enhanced apoptosis. Pharmacological induced reduction of DEPTOR and therefore mTOR-mediated interaction could lead to therapeutic benefits in MM treatment, as latest *in vivo* studies in DEPTOR knockout mice verified accelerated inhibition of mTORC1 [22, 44].

Concluding, tumor metabolism certainly underlies a complex network and therefore the universal and consistent validity of the Warburg effect has to be scrutinized and questioned. Smolková et al. for instance propose 2 metabolic phenotypes in cancer cells depending on their surrounding microenvironment: (i) increased glycolysis and increased mitochondrial OXPHOS in case of hypoxia and (ii) relatively suppressed glycolysis and restoration of mitochondrial OXPHOS with nutrient shortage because of high proliferation rates. This approach emphasizes, that mitochondrial respiration impairment is not a rigid feature of cancer cells and can possibly be abrogated – a phenomenon described as “reversed Warburg effect” [16, 45]. Nonetheless interfering in the Warburg effect by inhibiting appropriate targets within the complex pathway demonstrates an adjuvant therapy strategy in MM treatment and can optimize the effect mechanisms of commonly used drugs such as bortezomib.

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