Metabolic Imbalance Associated Mitophagy in Tumor Cells: Genesis and Implications

Madhuri Chaurasia[1,2,#], Shashank Misra[1,#], Anant N. Bhatt[1], Asmita Das[2], Bilikere Dwarakanath[1,*] and Kulbhushan Sharma[1,*]

[1]*Division of Radiation Biosciences, Institute of Nuclear Medicine and Allied Sciences, Delhi 110054, India*

[2]*Department of Biotechnology, Delhi Technological University, Delhi 110042, India*

Abstract: Emerging knowledge supports the notion that metabolic reprogramming facilitates the progression of many cancers and in some it could be initiated by mutations in genes related to mitochondrial function. While dysfunctional mitochondria plays a pivotal role in driving metabolic reprogramming, mitophagy that recycles damaged mitochondria by selective and organized degradation appears to be vital for sustaining carcinogenesis. Although the potential of targeting mitophagy as a therapeutic strategy has still remained elusive, poor prognosis and therapeutic resistance of highly glycolytic tumors suggest that inhibitors of mitophagy could be potential adjuvant in radio- and chemotherapy of tumors. We briefly review the current status of knowledge on the interrelationship between mitophagy and metabolic reprogramming during carcinogenesis and examine mitophagy as a potential target for developing anticancer therapeutics and adjuvant.

Keywords: Warburg, PARKIN, Oxidative stress, Metabolic Reprogramming, Calcium.

Mitochondria are considered to be the energy house of eukaryotic cells. To ensure functionality of this crucial requirement under a variety of stress conditions, cells have evolved a highly structured mechanism for recycling damaged mitochondria known as Mitophagy [1, 2]. Mitophagy aids in selective degradation of damaged/dysfunctional and old mitochondria produced in response to certain deleterious stresses such as hypoxia and starvation, thereby helping in the maintenance of cellular homeostasis [1, 2]. Accumulating evidences suggest that dysfunctional mitochondrion has a pivotal role in modulating the metabolic reprogramming thus contributing to the process of tumorigenesis [3]. Variations in the status of Warburg phenotype linked to the differences in mitochondrial status in cancer cells and/or tumor micro milieu (reverse Warburg phenotype) appear to be dependent on mitophagic potential of cells as well as the type and extent of stress [4]. Poor prognosis and therapeutic resistance of highly glycolytic tumors suggest that mitophagy could be one of the contributing factors. Although the potential of targeting mitophagy as a therapeutic strategy has so far remained elusive, emerging evidences suggest the potential of targeting this phenomenon for developing inhibitors of mitophagy as adjuvant in radio- and chemotherapy of tumors [5]. This review will discuss the relationship between metabolic disturbance leading to calcium imbalance and mitophagy in both malignant as well as untransformed cells and critically examine the direct and collateral evidences for developing inhibitors of mitophagy as adjuvant in cancer therapy.

METABOLIC REPROGRAMMING AND TUMORI-GENESIS

Metabolic reprogramming or altered bioenergetics has emerged as an important hallmark of cancer. The source of cancer initiation and maintenance which was earlier only restricted to genetic mutations is now gradually being attributed to metabolic reprogramming also. Glucose and ATP have been identified as key players in altered bioenergetics. Metabolic alternations in cancer cells were recognized as early as 1920, when Otto Warburg gave his hypothesis of "Warburg effect" stating that ''*Cancer, above all other diseases, has countless secondary causes".*

Warburg postulated that unlike normal cells, cancer cells produce lactate from glycolysis even in the presence of abundant oxygen. He termed it as aerobic glycolysis. Warburg attributed this phenomenon to dysfunctional mitochondria that impairs oxidative phosphorylation [3, 6]. High glycolytic rate might also result from a decreased mitochondrial mass in tumor cells [7]. The constant glycolysis in these cells is maintained by up regulation of glucose transporters (Glut1-4) that help in glucose uptake [8-11].

In addition to providing ATP, increased glucose uptake also provides cancer cells with building blocks of the cell i.e. macromolecules like nucleotides, amino

*Address correspondence to these authors at the Division of Radiation Biosciences, Institute of Nuclear Medicine and Allied Sciences, Delhi 110054, India; Tel: +91-11-23918838; Fax: +91-11-23919509;
E-mails: dwarakanathdrbs@gmail.com; kulsinmas@gmail.com

[#]These authors contributed equally.

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acids and lipids by diverting glucose to Pentose Phosphate Pathway [12-14]. The generation of biomass maintains rapid proliferation and provides a balance between the anabolic and catabolic activities of cells. Warburg effect also maintains the damaged reactive oxygen species (ROS) levels by generation of adequate NADPH *via* phosphoenol pyruvate pathway and through PKM2 isoform of the pyruvate kinase (PK), which catalyzes the conversion of phosphoenol pyruvate (PEP) to pyruvate as the last step of glycolysis. The PKM2 isoform also helps in maintaining rapid proliferation by up regulation of glucose transport and enhanced synthesis of early glycolytic intermediate in order to achieve metabolic balance among ATP production, biomass synthesis, as well as the control of oxidative stress due to ROS generation [15, 16].

Although Warburg's interpretation of the association of aerobic glycolysis with dysfunctional mitochondria in tumor cells has been challenged in recent times, subsequent studies revealed that tumor mitochondria do respire and produce ATP [17]. The Warburg effect specifically assigned to cancer cells has also been observed in rapidly proliferating normal cells such as stimulated lymphocytes and mitotic and proliferating fibroblasts [18-22]. Thus, it appears that phenomenon of aerobic glycolysis is a metabolically conserved process adapted to sustain growth of highly proliferating cells in order to fulfill their energy and metabolic demands. Impairment in growth of breast cancer even at high levels of glycolysis and promotion of tumorigenesis with enhanced basal autophagy leading to the maintenance of mitochondrial function in cells with activated Ras during periods of nutrient limitation suggest that aerobic glycolysis is not applicable to all cancer cells [23, 24].

MITOCHONDRIAL ALTERATIONS AND TUMORIGENESIS

Mitochondrial dysfunction has been implicated in the pathogenesis of the various disorders including Parkinson's disease, diabetes mellitus and cancer [25-30]. Polymorphism in mitochondrial DNA enhancing the susceptibility to breast and prostate cancer, and recent identification of fumarate and succinate dehydrogenases as tumor suppressor genes have highlighted the relationship between mitochondria and tumorigenesis [31-34]. Besides genetic changes, enhanced ROS production leading to oxidative stress, suppression of mitochondrial outer membrane potential (MOMP) (that elicits apoptosis) and enhanced glycolysis in cancer cells also indicate involvement of mitochondria in tumorigenesis even at the functional level [35-37].

Several evidences support the mitochondrial association with tumorigenesis at the genetic level. Polymorphism in mitochondrial DNA promotes tumorigenesis *via* two ways i.e. by impeding steady-state oxidative phosphorylation (OXPHOS) and by facilitating cancer cell adaptation to changing bioenergetics environments [38]. Further, mutations in the genes encoding proteins such as succinate and fumarate inhibits α-ketoglutarate-dependent prolyl hydroxylases (PHDs), thus stabilizing hypoxia-inducible factor 1α (HIF1α) [39]. The stabilized HIF1α is then translocated to the nucleus causing a shift in energy metabolism from oxidative to glycolysis [40, 41]. Fumarate inhibition also potentiates tumorigenesis by succinylation of cysteine residues in Kelch-like ECH-associated protein 1 (KEAP-1), which activates nuclear factor (erythroid-derived 2)-like 2 (NRF2) pathway thereby up-regulating the level of stress response genes [42, 43].

Generation of ROS leading to the altered cellular redox state by both functional and dysfunctional mitochondria is also known to promote tumorigenesis. Increased ROS disrupts mitochondrial signaling by oxidizing thiol groups in cysteine residues of caspases as well as cysteine residues of phosphotases like PTEN tumor suppressor, the CDC25B oncogene, and MAPK phosphatases [44-46]. Increased ROS also stabilizes HIF1α which in-turn impairs respiration (TCA cycle) leading to the reduction in ROS levels and thus protecting tumor cells from apoptosis [47-50]. Promotion of tumorigenesis by ROS is also evident by the degradation of the KEAP-1 that activates NRF2 signaling [51]. NRF2 pathway endorses metabolic programming towards anabolic pathways that sustains tumor growth along with maintaining damaged ROS levels that further potentiates tumor cell proliferation [52]. MOMP suppression has also been shown to promote tumor growth by inhibiting apoptosis of tumor cells [36]. Thus, accumulating evidences indicate a strong relationship between mitochondria and tumorigenesis both at the genetic as well as functional levels.

MITOCHONDRIAL ALTERATIONS INDUCED METABOLIC REPROGRAMMING

The mechanistic link between mitochondria and aerobic glycolysis is provided by Hexokinase II (HK-II) that gets up-regulated in cancer cells and translocates

to the voltage-dependent anion channel (VDAC) in the mitochondrial outer membrane. This interaction is facilitated by the phosphorylation of HK-II by protein kinase B (Akt) [53]. The mitochondrial HK-II being in close proximity to ATP source facilitates rapid phosphorylation of glucose leading to higher glycolysis and PPP. Another interconnecting link between Warburg effect and mitochondria is the pyruvate kinase M2 (PKM2), which maintains tumor growth by up-regulating HIF1α and ROS levels as well as providing biomass to the cancer cells [54]. However, this interaction is context and tumor-type dependent as under moderate hypoxia conditions, PKM2 is inhibited through its oxidation, leading to the promotion of PPP pathway and enhanced generation of cellular NADPH which prevents oxidative stress generation. On the other hand, during severe hypoxia, the dependency of cancer cells on PKM2 increases owing to the limited oxidative phosphorylation [54].

Tumor cells are heterogeneous in terms of metabolism and morphology. Metabolic heterogeneity includes variations in the levels of oxidative phosphorylation and Warburg effect due to fluctuations in the oxygen and nutrient supply. Besides the tumor cells, heterogeneity has also been shown in the stromal cells present in the tumor micro milieu consisting cells of hematopoietic (T cells, B cells, NK cells, macrophages and MDSC) and mesenchymal origin (fibroblasts, myofibroblasts, mesenchymal stem cells (MSCs), adipocytes and endothelial cells) [55]. However, the role of these cells in metabolic reprogramming of tumor cells has remained elusive. Recently, a new concept of “Reverse Warburg effect” or “Battery-operated tumor growth” (hereafter called as non-Warburgian phenotyope) has been proposed where the stromal cells appear to influence the metabolic reprogramming of tumor cells through a host-parasite relationship, with stromal cells acting as host and cancer cells as parasites [4]. The stromal cells surrounding tumor cells have also been shown to display efficient mechanism for recycling dysfunctional mitochondria acting as a nutrient supplier [4]. However, the implications of efficient recycling of mitochondria in the tumor cells and micro milieu on the resistance against chemo- and radiotherapies have not been well understood.

ROLE OF CALCIUM IN FUNCTIONAL ALTERATION OF MITOCHONDRIA

Hypoxia and/or altered metabolism are the major sources of oxidative stress in cancer cells. This persistent oxidative stress leads to the chain reaction of cellular lipid oxidation. Oxidized lipid metabolites (by-products) either alter the membrane fluidity or get released inside the cytoplasm and the respective organelles. The oxidized lipids alter the permeability of membrane to calcium or directly act as calcium ionophores leading to increased cytosolic calcium [56, 57]. Mitochondria buffer this overloaded cytosolic calcium by acting as a sink thus preventing cell death. However, calcium accumulation in mitochondria leads to hormesis effect, called mitohormesis [58, 59]. At low concentration, calcium enhances the oxidative phosphorylation capacity by activating many mitochondrial dehydrogenases leading to aggressive metabolic phenotype [58]. Majority of the cancer cells show mitochondrial accumulation in the close proximity of ER, creating the microdomain of high calcium for mitochondrial calcium uniporter leading to regulated increase in mitochondrial calcium, thus assisting in the development of the aggressive metabolic phenotype for enhanced growth and survival [60]. On the other hand, calcium overload in the mitochondria leads to mitochondrial damage [56].

Since the accumulation of damaged mitochondria is detrimental to cells and one of the major causes of cancers, mitochondrial quality control is essential for maintaining the cellular integrity and function [61]. Therefore, damaged and functionally compromised mitochondria undergo the process of degradation and regeneration of newer mitochondria called mitophagy and mitochondrial biogenesis respectively. As oxidative stress and calcium induced mitochondrial damage is continuous process in cancer cells, damaged mitochondria can be observed in them at any given time in the form of mitochondria derived vesicles (MDVs) and “I-Bodies” [56, 61, 62]. Taken together, all these events appear to be inter-dependent and work in a cyclic manner in the cancer cell. Oxidative stress leads to disturbance in cellular calcium homeostasis causing mitochondrial damage and altered metabolism, further resulting in to enhanced ROS production in cancer cells (Figure **1**).

MITOPHAGY: RECYCLING OF THE DAMAGED MITOCHONDRIA

Mitochondrial dysfunction has been shown in numerous patho-physiologcal conditions including cancer, metabolic disorders, neurodegeneration, diabetes and aging [1, 2, 63]. Being a critical component, various mitochondria quality control mechanisms exist in cells that include mitochondrial

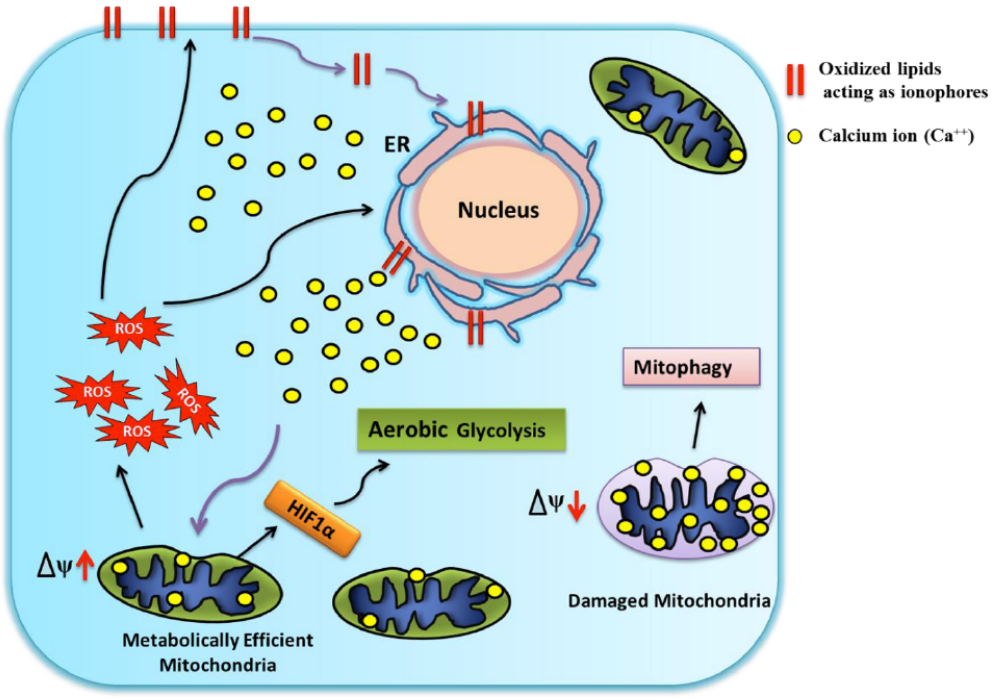

Figure 1: Schematic diagram showing oxidative stress induced alterations in calcium homeostasis during metabolic reprogramming of cancer cells. ROS induced lipid oxidation makes membrane permeable to calcium, which is buffered by mitochondria making it metabolically efficient. This leads to further ROS production and stabilization of HIF1α converting the cancer cell in to Warburg phenotype. Mitochondria overloaded with calcium develop irreversible damage and are cleared from cells through mitophagy.

fusion, fission, biogenesis and mitophagy (mitochondrial autophagy). Mitophagy is the primary mechanism responsible for the recycling of damaged and dysfunctional mitochondria with the help of autophagosome, which further fuses with lysosomes to form autophagolysosomes [64, 65]. Mitophagy and mitochondrial fusion are antagonistic, and decide the fate of dysfunctional mitochondria [66]. Mitochondrial fission takes place predominantly in the depolarized mitochondria lacking fusion protein optic atrophy 1 (Opa-1), whereas mitochondrial fusion takes place in polarized mitochondria *via* depletion of mitochondrial fission protein dynamin-related protein 1 (Drp1) with the help of protein kinase A (PKA) [67-72]. As mitochondria cannot be recycled in its original form due to its large size, mitochondrial fission is considered as the prerequisite for initiating mitophagy.

During mitophagy, numerous key adaptor molecules at the outer membrane of damaged and dysfunctional mitochondria, facilitates interaction with LC3 (autophagy related protein which helps in autophagosome elongation) present at the growing autophagosome membrane [64, 73]. The main adaptors involved in mitophagic induction include E3 ubiqutin ligase PARKIN, NIX, BNIP3, FUNDC1 and Mul1 [64, 73, 74]. Oxidative phosphorylation in damaged mitochondria leads to the generation of toxic by-products involving reactive oxygen species (ROS), which causes oxidative damage to mitochondrial lipids, DNA and proteins leading to further ROS production. The damaged mitochondria in turn, release huge amount of Ca^{2+} ions and cytochrome-c to the cytosol thereby triggering apoptosis [75-77]. Although specific mechanisms involved in mitophagic induction are not completely understood, two molecular pathways have been implicated. The first pathway depends upon PINK1 (PTEN induced putative kinase 1) and PARKIN interaction where PINK1 is a mitochondria specific kinase and PARKIN is an E3- ubiquitin ligase [78]. The second is mainly mediated *via* different molecules such as ER associated E3 ubiquitin ligase GP78 (glycoprotein 78) and NIX/BNIP3L in a context dependent manner [78, 79].

Accumulating evidences suggest the involvement of nearly 32 autophagy related (ATG) proteins in mitophagic progression. A new autophagy related protein i.e. ATG33, which is specifically involved in mitophagy has been recently identified as a mitochondrial outer membrane protein [80, 81]. The core mitophagic machinery is activated by the recruitment of ATG32-ATG11-ATG8 (LC3 in mammals), where ATG11 acts as an adaptor between

ATG8 and ATG32 and helps in the recruitment of autophagic machinery over mitochondria. Under normal conditions, PINK1 (63 kDa) gets translocated from outer mitochondrial membrane to the inner mitochondrial membrane where PARL (presenselin associated, rhomboid-like) protease causes its proteolytic cleavage into a short PINK1 isoform of approximately 52 kDa ($PINK1_{52}$) [82, 83]. However, under conditions of reduced mitochondrial potential (like oxidative stress), PINK1 accumulates in the outer mitochondrial membrane, where it interacts with PARKIN and causes its phosphorylation [84]. Activated PARKIN causes ubiquitination of various mitochondrial proteins, which act as a landing platform for p62/SQSTM1 forming a functional link between ubiquitinated proteins, including MFN1/2 (Mitofusin1/2) and LC3, leading to the initiation of autophagosome with the help of Atg32 (Figure **2**).

MITOPHAGY AND CANCER

During trauma, mitophagy supports tumor cell survival by providing substrates for mitochondrial metabolism [24, 85]. Aggressive tumor cells appear to harbour robust mitochondria, although due to severe mutations in tumor suppressor as well as TCA cycle genes, they rely more on aerobic glycolysis to meet their energy demands. Such mitochondria show 'Warburg phenomenon'. In addition to the mutations in metabolic regulatory genes, several mitophagy related genes have also been found to be mutated in many types of cancers during initial stages of tumor development [86, 87]. This results in the induction of defective mitophagy in these cells, leading to a higher accumulation of dysfunctional mitochondria ultimately leading to enhanced ROS generation and tumor induction [85]. Such mitochondria are more robust, having high antioxidant defence mechanism and can survive in highly hypoxic environment. We recently showed that Ca^{2+} rich structures formed by high intracellular Ca^{2+} induced dysfunction and aggregation of mitochondria in response to stress can be revealed by high density packing of the fluorescent calcium ionophore A23187 called "I-Bodies" [56]. Presence of endogenous "I-Bodies" in cancer cells indeed supports the association of mitochondrial dysfunction and carcinogenesis [56]. "I-Bodies" are suggested to provide a snapshot view of the ongoing mitophagy or genetic defect in the clearance of dysfunctional mitochondria in cancer cells similar to Parkinson's disease [56, 88]. Increase in radiation and anticancer drug (etoposide) induced "I-Bodies" validate the hypothesis that "I-Bodies" are mitophagic vacuoles encircling damaged mitochondria.

Cellular compositions of tumors are highly heterogeneous, with clonal variations of tumor cells

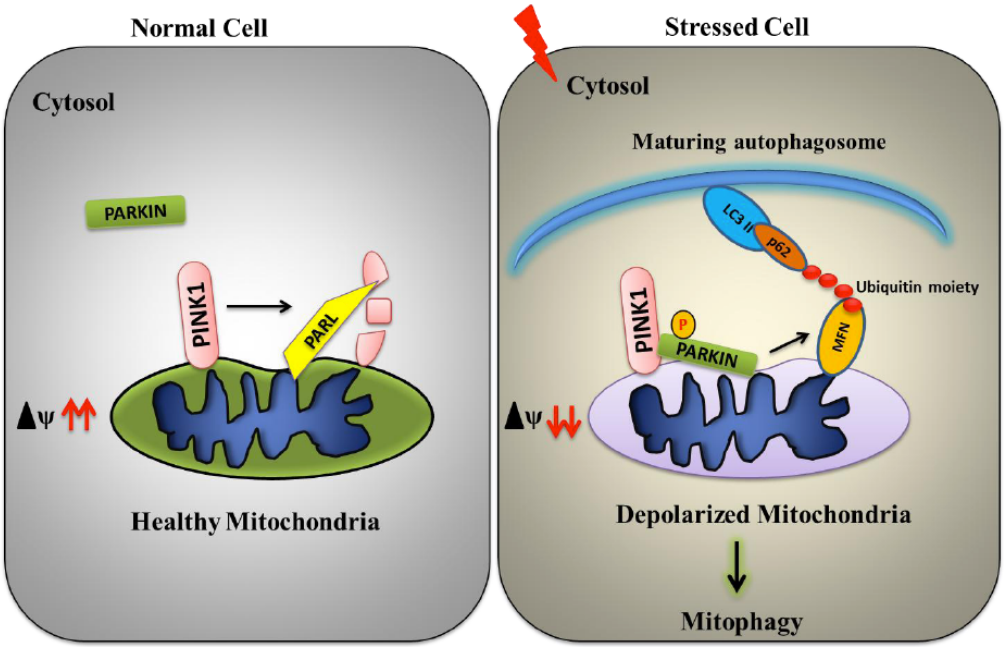

Figure 2: Mechanism of mitophagy induction. In normal cells, onset of mitophagy is abrogated by the proteolytic cleavage of PINK1 by PARL. On exposure to stress, same cell undergoes mitophagy in a sequential manner. Reduction in the mitochondrial membrane potential leads to the induction of mitophagic calcium *via* accumulation of PINK1, facilitating translocation and activation of PARKIN that adds ubiquitin moiety to mitochondrial fusion proteins Mfn, thereby inhibiting mitochondrial fusion. PINK1-PARKIN interaction initiates the mitophagic process *via* engulfment of damaged mitochondria.

and other tumor-associated cell types including fibroblasts, endothelial and immune cells. These cells constitute the tumor stroma and have also been shown to display efficient dysfunctional mitochondria recycling which acts as nutrient supplier thus fertilizing the tumor niche and thereby helping in tumor progression and resistance [1, 2, 63]. However, the effects of the efficient recycling of mitochondria (mitophagy) in tumor cells/micro milieu on their resistance against chemo- and radiotherapies have not been clearly understood.

The mitophagy related protein PARKIN has been identified as a p53 target gene and has been reported to prevent the Warburg effect by encouraging oxidative metabolism [87]. PARKIN has also been found to be deleted in numerous cancer conditions namely ovarian, lung, and breast cancer [89, 90]. Further, mice with severe PARKIN mutations have been found to be more vulnerable to spontaneous liver tumors [86, 87]. Mutations in other mitophagy related adaptor proteins like BNIP3 and NIX enhances tumor invasiveness and malignancies in lung, colorectal, hematologic, liver, and pancreatic cancers [91-97]. Thus, these studies suggest an inverse relationship between initiation, progression and resistance to the therapies vis-a-vis the mitophagy potential of tumors. In contrast, mitophagy has also been shown to be a tumor-promoting process which is supported by its ability to maintain a healthy mitochondrial pool required to fulfil the enhanced energy need of tumor cells [24, 98].

TUMOR ASSOCIATED MITOPHAGY AND AEROBIC GLYCOLYSIS

Although not well established, circumstantial evidences indicate a direct relationship between tumorigenesis and mitophagy [99, 100]. Similar to autophagy, mitophagy is also involved in maintaining functional (and thus energy generating) mitochondrion pool as well as nutrients for better cancer cell survival. A direct relationship between mitophagy and glycolysis is still lacking. Available evidences suggest that as functional mitochondria are a prerequisite for energy generation through glycolysis in a tumor cell (Warburg effect), mitophagy must add on to the survival and progression of tumorigenesis even during therapeutic stress [101, 102]. For instance, Ras oncogene positive tumors have been shown to activate mitophagy which is associated with enhanced glycolysis [103].

The impact of alterations in metabolic reprogramming and mitophagy in the tumor micro milieu has been recently explored. Many tumor cells appear to maintain their mitochondrial function of enhanced glycolysis *via* a complex mechanism wherein tumor cells indirectly derive energy from the neighbouring cells in the tumor microenvironment; the tumor stromal cells which exhibit a higher glycolytic phenotype i.e. Warburg Effect [4]. As a messenger, tumor cells generate enormous amounts of reactive oxygen species (ROS), which gets released into the tumor micro milieu. Tumor stromal cells gets influenced by this huge ROS supply, thus initiating the onset of stromal oxidative stress, autophagy and mitophagy due to the activation of key transcription factors, namely HIF1α (aerobic glycolysis) and NFkB (inflammation) [104-111]. Two types of mitochondria may exist in these stromal cells; those which are less robust and signal mitophagy initiation on sensing the ROS released into the micro environment followed by their altered membrane potential (Non-Warburgian), and those which are more robust and start L-lactate production after sensing oxidative stress (Warburgian). Mitophagic degradation of non-Warburgian mitochondria provides recycled products as well as raw materials for the Warburgian mitochondria to facilitate aerobic glycolysis and enhanced tumor stromal lactate production. This lactate produced by Warburgian mitochondria is released into the tumor microenvironment with the help of mono-carboxylate transporter 4 (MCT4) and MCT1 [112, 113]. In response to the nutrient (in form of lactate) released into the micro milieu, cancer cells exhibit 'reverse Warburg phenomena' where L-lactate functions as an onco-metabolite, stimulating mitochondrial biogenesis, glutaminolysis and OXPHOS in them, thereby directly providing energy for their growth and mitochondrial biogenesis [114]. In contrast, stromal cells have also been associated with tumor regression and tumor cell killing. Stromal cells of hematopoietic origin such as T cells, dendritic cells and NK cells have been found to suppress tumor progression and therefore projected as targets for developing anti-tumor therapeutics [115-118].

Cancer associated fibroblasts have also been shown to over express mitochondrial fission factor (MFF) which is considered as the prerequisite for mitophagy [119, 120]. The MFF over-expressing fibroblasts undergo oxidative stress with augmented ROS production and NF-kB activation, thus driving the onset of mitophagy and ultimately, glycolytic metabolism [120]. Similarly, MFF has been shown to promote a glycolytic phenotype *in vivo*, under conditions of hypoxia, where cancer associated

fibroblasts (MFF fibroblasts) become more glycolytic and display an efflux of high-energy mitochondrial fuels into the extracellular microenvironment which help drive mitochondrial biogenesis in cancer cells.

Mitophagy and glycolysis show strong interrelationship in stromal cells as well as cancer cells thereby promoting tumor cell survival even under adverse conditions of therapy [121]. Therefore, mitophagy appears to be a key quality control deciding the response of cancer cells to therapy and may thus be a potential target for adjuvant therapy.

THERAPEUTIC IMPLICATIONS OF TARGETING MITOPHAGY

Application of mitophagy inhibitors as primary or adjuvant tumor therapy has not yet been translated to the clinics. However, emerging knowledge suggests a potential for developing therapeutic strategies targeting mitophagy [122]. Inhibitors of glycolysis like 2-deoxy glucose (2-DG) and 3-bromopyruvate have been shown to selectively induce tumor cell death as well as enhance death induced by anticancer therapies like ionizing radiation and chemotherapeutic drugs [123-125]. However, a great deal of heterogeneity has been observed in both these effects among well-established tumor cell lines *in vitro*, animal tumors *in vivo* and clinical response [123, 126, 127]. This heterogeneity may be partly attributed to the presence of both Warburgian as well as non-Warburgian mitochondria in resistant tumors [4]. Mitophagy as well as enhanced glycolysis in these non-Warburgian mitochondria assists in providing nutrients to the Warburgian phenotype, thereby augmenting the tumor resistance. Thus, inhibition of mitophagy in combination with metabolic modifiers (like 2-deoxy-glucose, metformin etc.) can be a potential approach for improving the efficacy of radio- and chemotherapies (Figure **3**).

To what an extent variations in the treatment induced mitophagy (or autophagy) contributes to the heterogeneous responses observed in pre-clinical and clinical studies needs further investigations using genetically modified cell systems. Combinations of antioxidants like N- acetyl cystein and quercetin which can inhibit mitophagy as well as lactate production leading to the accumulation of more dysfunctional mitochondria ultimately driving the cell towards apoptosis could also be a potential strategy that requires systematic investigations [128, 129]. Furthermore, inhibitors of mitochondrial fission that inhibit mitophagy in stromal as well as tumor cells could also be potential adjuvants.

Mitophagy exhibits a double faceted role in tumorigenesis i.e. either survival-supporting or death-promoting [121, 128, 129]. Therefore, inducing prolonged or robust mitophagy using mitophagy modifiers along with the conventional anti-cancer therapies could also be explored as an anti-cancer strategy. Prolonged mitophagy in tumor cells would exhaust the metabolites required for sustaining the tumor growth ultimately leading to cell death. Induction of robust mitophagy using linamarase/linamarin/ glucose oxidase (lis/lin/GO) system leading to the loss of mitochondrial membrane potential and irreversible cell death of tumor cells has been reported recently. Similarly, induction of mitophagy by ceramide; and enhanced cell death of nasopharyngeal carcinoma (CNE2) during low-intensity ultrasound therapy in the presence of curcumin on induction of mitophagy further substantiate the potential of targeting robust or treatment induced prolonged mitophagy [130-132]. Various anticancer agents like ionophores and drugs which alter mitochondrial permeability transition pores (mPTPs) such as paclitaxel and doxorubicin that induce apoptosis, have been shown to enhance mitophagy and autophagy [133-135]. These observations suggest induction of mitophagy as an attractive anticancer approach. Further, administration of glycolytic inhibitors in combination with mitophagy inducing chemotherapies have been proposed to significantly enhance tumor cell death as a result of increased dependency of tumor cell on glycolysis following excessive mitophagy [5]. This also explains the enhanced efficacies of mPTP opening drugs when administered with glycolytic inhibitors like lonidamine (a hexokinase inhibitor) [136, 137]. Even though induction of prolonged or robust mitophagy appears reasonable, care must be taken as robust induction would depend upon the type and degree of stress. Moreover, the specificity of these approaches towards tumor cells needs to be investigated further.

The lack of specific biomarkers and understanding of the mitophagy associated tumor cell death is another hurdle that needs to be considered in order to make this strategy feasible in the clinics. Association of Glut-4 and over-expression of MCT as well as deletion in Caveolin-1 have been shown in resistant and aggressive tumors [138-140]. Since these are associated with reverse Warburgian phenotype as well as enhanced mitophagy, they may serve as markers for identifying tumors where mitophagy inhibitors could be useful in combination with other therapeutic agents. Since host factors also contribute to the responses of

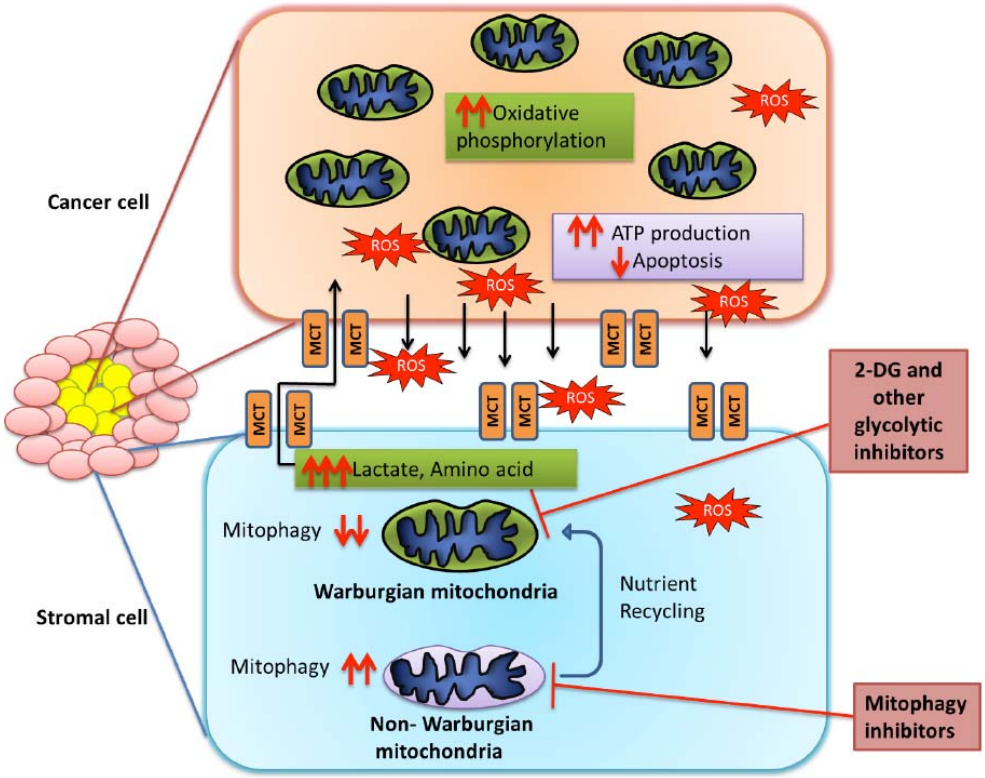

Figure 3: Host parasite relationship between stromal and cancer cells and the influence of metabolic reprogramming in tumorigenesis. The stromal cells (host) that display both Warburg & non-Warburgian mitochondria provide nutrition and biomass to the cancer cells (parasite) for their growth. Potential targets for developing therapeutics/adjuvant (with currently known inhibitors) are also shown.

tumors under *in vivo* conditions, identification (establishment) of appropriate surrogate markers will be helpful in individualizing therapies targeting mitophagy/autophagy for improving therapeutic gain.

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