

Metabolic alterations accompanying oncogene-induced senescence

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Abbreviations: 3-AP, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone; ACC, acetyl-CoA carboxylase; ACLY, ATP-dependent citrate lyase; CPT1, carnitine-palmitoyl transferase I; dNDP, deoxyribonucleoside diphosphate; dNTP, deoxyribonucleotide triphosphate; FASN, fatty acid synthase; GPC, L- α -glycerophosphocholine; MDH, malate dehydrogenase; ME, malic enzyme; NAD, nicotinamide adenine dinucleotide; NDP, ribonucleoside diphosphate; NTP, ribonucleoside triphosphate; OIS, oncogene-induced senescence; PC, phosphocholine; PDH, pyruvate dehydrogenase kinase; PDK, pyruvate dehydrogenase kinase; PDP2, pyruvate dehydrogenase phosphatase 2; PKM2, pyruvate kinase M2; PML, promyelocytic leukemia; PPAR, peroxisome proliferator-activated receptor; PPP, pentose phosphate pathway; SA-B-Gal, senescence-associated β -galactosidase; RISP, Rieske iron sulfur protein; RNR, ribonucleotide reductase; ROS, reactive oxygen species; RRM1, ribonucleotide reductase subunit M1; RRM2, ribonucleotide reductase subunit M2; RRM2B/p53R2, ribonucleotide reductase subunit M2B; SASP, senescence-associated secretory phenotype; SDHA, succinate dehydrogenase; TCA, tricarboxylic acid.

Senescence is defined as a stable cell growth arrest. Oncogene-induced senescence (OIS) occurs in normal primary human cells after activation of an oncogene in the absence of other cooperating oncogenic stimuli. OIS is therefore considered a *bona fide* tumor suppression mechanism *in vivo*. Indeed, overcoming OIS-associated stable cell growth arrest can lead to tumorigenesis. Although cells that have undergone OIS do not replicate their DNA, they remain metabolically active. A number of recent studies report significant changes in cellular metabolism during OIS, including alterations in nucleotide, glucose, and mitochondrial metabolism and autophagy. These alterations may be necessary for stable senescence-associated cell growth arrest, and overcoming these shifts in metabolism may lead to tumorigenesis. This review highlights what is currently known about alterations in cellular metabolism during OIS and the implication of OIS-associated metabolic changes in cellular transformation and the development of cancer therapeutic strategies.

Introduction

Senescence

Senescence was first described in 1961 by Leonard Hayflick and Paul Moorhead while working at The Wistar Institute in

Philadelphia.¹ Specifically, these scientists found that normal cells in culture could only double a limited number of times, after which the cells would exit the cell cycle (termed “senescence”). In the decades since this discovery, a number of other stimuli have been shown to induce senescence, including oncogene activation, DNA damage, oxidative damage, and certain chemotherapeutic agents.² Although the cells do not continue to replicate their DNA, it is well appreciated that senescent cells remain metabolically active, and that these metabolic changes play a role in both senescence-associated cell growth arrest and human disease.

Oncogene-induced senescence

Oncogene-induced senescence (OIS) occurs when an oncogene (such as RAS, BRAF, MYC, etc.) becomes activated in a normal diploid cell.³ This leads to a paradoxical stable cell cycle exit, which arrests cell growth.⁴ Therefore, OIS is considered a *bona fide* tumor suppressor mechanism *in vivo*.^{4,5} For instance, benign nevi, which are often characterized by mutations in BRAF and NRAS, have been shown to be senescent lesions.⁴

OIS is characterized by a number of phenotypic and molecular alterations. Phenotypically, cells that have undergone OIS exhibit a large, flat morphology and are positive for increased β -galactosidase activity (termed senescence-associated β -galactosidase [SA- β -Gal]).⁶ Additionally, OIS is often accompanied by accumulation of DNA damage, in particular DNA double strand breaks, and a robust DNA damage response.⁷⁻¹⁰ This damage is largely due to the aberrant DNA replication observed during OIS.^{9,11} Further, OIS is characterized by upregulation of the p53/p21 and pRb/p16 pathways,¹² which both play a role in inhibition of cell cycle progression. Many recent reports demonstrate that cells undergoing OIS secrete a number of inflammatory mediators, including cytokines and chemokines, termed the senescence-associated secretory phenotype (SASP).¹³ The SASP

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is thought to play a role in both the establishment and maintenance of the senescence-associated cell growth arrest.¹⁴ Taken together, cells undergoing OIS activate a number of cellular signaling pathways to ensure a stable cell growth arrest.

Although senescent cells do not replicate their DNA, they remain metabolically active.^{2,15} It is now known that a number of changes in cellular metabolism occur during OIS.¹⁶ This review will focus on the changes in cellular metabolism that accompany OIS, including changes in nucleotide, glucose, mitochondrial, and lipid metabolism and autophagy. We will also discuss how these pathways may be circumvented during transformation and tumorigenesis.

Metabolic Changes During OIS

Nucleotide metabolism is decreased during OIS

Deoxyribonucleotide triphosphates (dNTPs) are necessary for both nuclear and mitochondrial DNA replication and repair.^{17,18} Alterations in dNTP levels are known to play a pathogenic role in a number of human diseases, including cancer.^{9,11} dNTPs can be synthesized through the *de novo* pathway or the salvage pathway.¹⁹ The rate-limiting step in the synthesis of dNTPs during the *de novo* pathway is reduction of ribonucleoside di- or tri- phosphates (NDPs/NTPs) to deoxyribonucleotide di- or tri- phosphates (dNDPs/dNTPS) by ribonucleotide reductase (RNR).^{17,18} RNR is a tetrameric complex comprised of 2 large catalytic subunits (R1: ribonucleotide reductase M1 [RRM1]) and 2 small regulatory subunits (R2: ribonucleotide reductase M2 [RRM2] or RRM2B/p53R2). Although RRM1 is expressed during all phases of the cell cycle, RRM2 and p53R2 are cell-cycle regulated.²⁰ RRM2 is expressed during S phase, when dNTPs are needed for DNA replication,²⁰ whereas p53R2 is expressed during G0/G1 and is important for DNA repair and mitochondrial DNA synthesis.²¹ Changes in the expression or activity of RNR can therefore lead to altered DNA synthesis and repair.

Our group and others have shown that the levels of all 4 dNTPs are significantly decreased during OIS.^{9,11,22} We found that this is specifically due to a decrease in expression of RRM2 (Fig. 1), but not RRM1 or p53R2.⁹ Interestingly, the decrease in dNTP levels via suppression of RRM2 is a driver, not a consequence, of the cell cycle exit.⁹ Increasing dNTP levels by ectopic expression of RRM2 or addition of exogenous nucleosides in oncogene-expressing normal diploid cells is able to overcome the aberrant DNA replication, DNA damage accumulation, and senescence-associated stable cell growth arrest induced by oncogenic RAS or BRAF.^{9,22} Additionally, decreasing nucleotide metabolism using short hairpin RNA-mediated specific knockdown of RRM2 expression can induce the senescence-associated stable cell growth arrest via aberrant DNA replication and DNA damage accumulation.⁹ These results demonstrate that nucleotide metabolism is decreased during OIS and is a key driver of the OIS phenotype.

We recently reported that RRM2, and therefore nucleotide metabolism, is decreased in human benign nevi with mutations in BRAF or NRAS.⁹ Decreased RRM2 expression was significantly correlated with increased p16 expression, suggesting that these cells have undergone OIS. Additionally, melanoma cell

lines and primary melanoma samples with mutations in BRAF or NRAS display a marked increase in RRM2 expression. This suggests that overcoming the suppression of nucleotide metabolism that occurs during OIS may allow cells to re-enter the cell cycle, gain additional mutagenic hits, and become transformed. Indeed addition of exogenous nucleosides or ectopic RRM2 could overcome full senescence induced by RAS.⁹ Additionally, supraphysiological levels of RRM2 or dNTPs can lead to DNA damage and genomic instability,^{9,23} which is a hallmark of cancer cells.^{24,25} RRM2 is transcriptionally regulated by E2F1 (Fig. 1).^{9,26,27} E2F1 is negatively regulated by pRb (Fig. 1),^{28,29} which is an important effector of the senescence phenotype.¹² In some contexts, loss of pRb can suppress the OIS phenotype.¹² When pRb is lost, E2F1 is no longer repressed and can induce transcription of a number of genes that regulate metabolism, including RRM2.³⁰ Additionally, loss of p53 can suppress OIS and can overcome OIS in cells with low p16 expression.^{10,31,32} Interestingly, a repressive E2F, E2F7, is a p53 target gene.³³ E2F7 cooperates with pRb to promote senescence, thus coupling the p53 and pRb pathways. We have previously shown that E2F7 replaces E2F1 on the RRM2 promoter during OIS to suppress RRM2 transcription (Fig. 1).⁹ This suggests that p53 and pRb act in concert to suppress nucleotide metabolism during OIS, and loss of these tumor suppressors may overcome OIS due to an increase in nucleotide metabolism, thereby leading to cancer formation.

Glucose metabolism is altered during OIS

Glucose is one of the main cellular nutrients and is used in a variety of cellular metabolic processes such as the pentose phosphate pathway (PPP) and the tricarboxylic acid (TCA) cycle.^{34,35} Glucose metabolites are necessary for nucleotide, amino acid, and lipid biogenesis. Under normal conditions, cells take up glucose through glucose transporters. Intracellular glucose is then metabolized during a process called glycolysis, which has an end product of pyruvate and produces 2 ATP molecules. The pyruvate can then be further metabolized. In normal cells under anaerobic conditions, pyruvate is generally metabolized into lactate (fermentation), during which reduced nicotinamide adenine dinucleotide (NAD⁺) is produced. This NAD⁺ is important for cells to continue glycolysis. Under aerobic conditions, pyruvate dehydrogenase (PDH) metabolizes pyruvate into Acetyl Co-A to be further used in the TCA cycle and oxidative phosphorylation to produce 36 ATP molecules.

Cancer cells preferentially use glycolysis and subsequent fermentation to produce ATP even under aerobic conditions.^{24,34,36} First observed by Otto Warburg in 1924,³⁷ this is now known as the Warburg effect. Although glycolysis is a much less efficient way of producing ATP, cancer cells have a high demand for biomass to continue proliferation.³⁶ To keep up with both the bioenergetic and biomass needs of a highly proliferative state, tumor cells increase their glucose uptake and metabolism through a variety of mechanisms, including increased expression of glucose transporters and glycolytic enzymes.^{34,35} This allows for the high rate of growth and proliferation that is a hallmark of cancer cells.²⁴

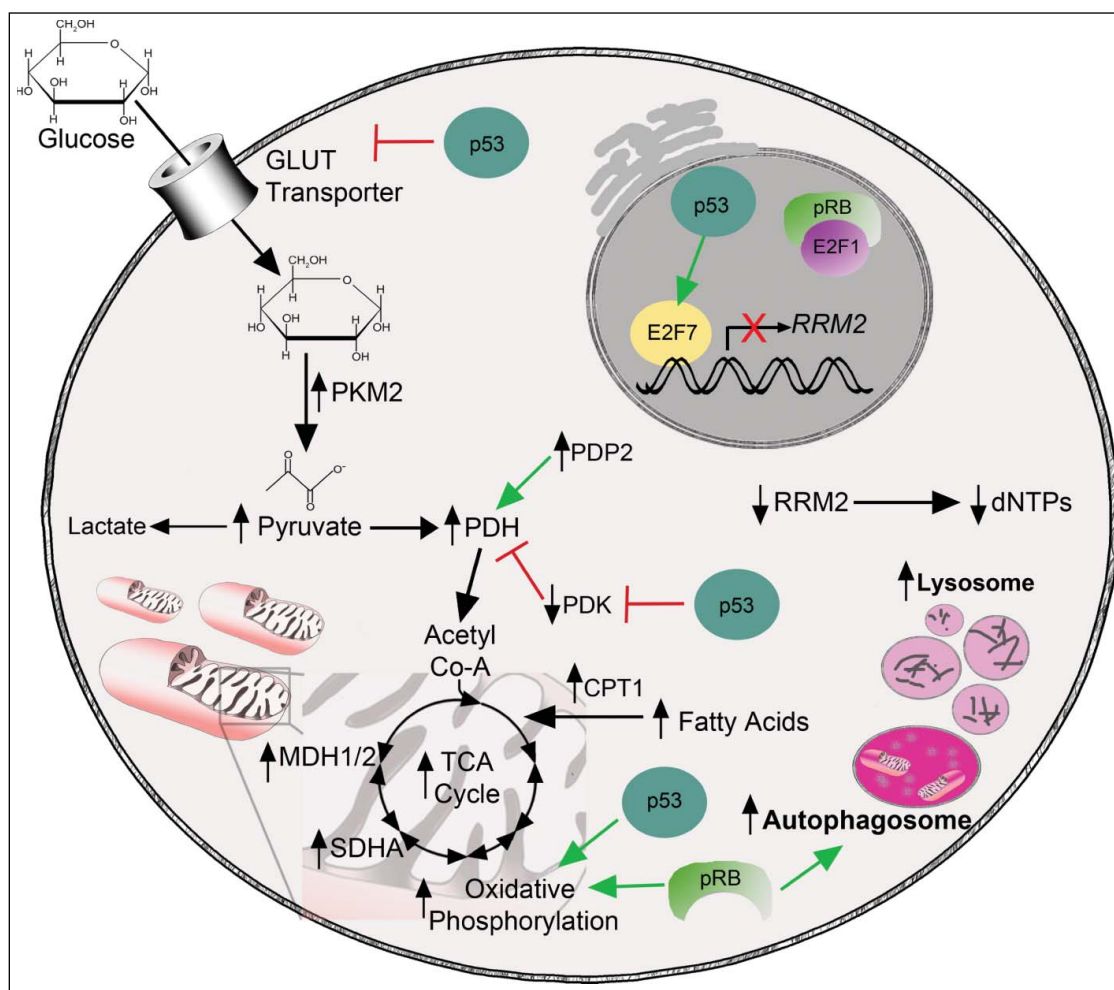


Figure 1. Alterations in metabolic pathways during oncogene-induced senescence. Glucose enters the cell through glucose transporters. p53 inhibits glucose uptake via negative regulation of glucose transporters.⁴⁵ During glycolysis, glucose is metabolized into pyruvate. One glycolytic enzyme, PKM2, is significantly upregulated during OIS.⁴⁰ Additionally, pyruvate levels are higher in cells that have undergone OIS.⁴⁰ The pyruvate produced by metabolism of glucose can be shunted into multiple pathways including fermentation, which leads to lactate production, or into the TCA cycle after further processing by PDH. PDH and its positive regulator PDP2 are upregulated during OIS, whereas the negative regulator of PDH (PDK1) is downregulated,^{42,43} possibly in part through inhibition mediated by p53.⁵³ This suggests that pyruvate is preferentially shunted into the TCA cycle and away from fermentation. Levels of TCA cycle metabolites are increased during OIS, as are a number of TCA cycle enzymes, including SDHA and MDH1/2.⁴³ Oxidative phosphorylation is also increased during OIS through increased expression of all complexes in the electron transport chain.⁴³ p53 and pRb also positively regulate oxidative phosphorylation.^{30,53,54} Nucleotide metabolism is decreased in cells that have undergone OIS as a result of suppression of E2F1-mediated transcription of RRM2 and increased repression by the p53 target E2F7.⁹ Free fatty acid levels are increased in cells undergoing OIS, most likely due to an increase in fatty acid oxidation.⁵² Finally, both autophagy and lysosomal activity are increased during OIS,^{68,75,76} which may be in part occur via positive regulation through pRb.⁷⁹

A number of recent studies have indicated that glucose metabolism is altered during OIS but with conflicting findings. Some studies have indicated an increase in glucose uptake during OIS.³⁸⁻⁴⁰ However, a number of other studies have observed either no change or a significant decrease in glucose uptake.⁴¹⁻⁴³ The basis for the discrepancy among these studies remains to be determined, although it may be due to differences in cell type, oncogenes, or timing. Regardless, all of these studies have shown changes in glucose metabolism when cells undergo OIS.

Two reports have demonstrated that cells undergoing OIS have increased pyruvate levels,^{40,42} demonstrating an increase in

glycolysis. This is most likely due to increased expression of a number of enzymes involved in pyruvate synthesis, such as pyruvate kinase (PKM2),⁴⁰ and pyruvate metabolism, including the gatekeeper enzyme pyruvate dehydrogenase (PDH) (Fig. 1).^{42,43} Because PDH expression is increased, the pyruvate that is produced during glycolysis is shunted into the TCA cycle, thereby decreasing shunting into the fermentation arm that leads to lactate production. Taken together, these studies reveal an overall shift in glucose metabolism in cells undergoing OIS toward the mitochondrial TCA cycle and away from fermentation and the Warburg effect. This is consistent with the fact that OIS is a tumor suppression mechanism.

It is thought that the Warburg effect is a consequence of increased cellular proliferation during tumorigenesis, and not necessarily a cause of transformation itself.⁴⁴ However, a number of important senescence effectors are known to play an important role in metabolism. For instance, loss of p53 can suppress OIS in some contexts.^{10,31,32} p53 is known to transcriptionally repress glucose transporters (GLUT-1, GLUT-3, GLUT-4) and a number of glycolytic enzymes.⁴⁵ Additionally, p53 positively regulates oxidative phosphorylation (Fig. 1),⁴⁶ thus loss of p53 can lead to a switch from oxidative phosphorylation to increased aerobic glycolysis.⁴⁶ Therefore, suppressing OIS could lead to a change in glucose metabolism that primes cells to become cancerous.

Mitochondrial metabolism is altered during OIS

Mitochondria are the cell's powerhouses. Under normal oxygen conditions, glucose is broken down into pyruvate, which is then shunted through the TCA cycle. The TCA cycle starts when pyruvate dehydrogenase metabolizes pyruvate into acetyl-CoA.⁴⁷ Acetyl-CoA can also be produced from fatty acid oxidation (discussed below).⁴⁸ A series of enzymatic reactions then occurs to produce 3 molecules of NADH, which can then fuel oxidative phosphorylation (oxphos). Oxphos occurs in the inner mitochondrial membrane through 5 enzyme complexes⁴⁹ and is the most efficient way for a cell to produce ATP as it generates 36 ATP molecules for each glucose molecule.³⁶

A number of recent studies have indicated a significant shift in mitochondrial metabolism in OIS cells. Firstly, a number of studies have demonstrated that cells undergoing OIS display mitochondrial dysfunction. Studies of RAS- or HER2-induced senescence reveal that cells undergoing OIS have decreased mitochondrial membrane potential,^{39,50} indicating mitochondrial dysfunction. Interestingly, these studies report conflicting results in terms of mitochondrial mass and mitochondrial DNA content, likely due to differences in cell type and the oncogene used. Cadenas et al. reported that mitochondrial mass is decreased and that mitochondria are found in vacuole-rich areas, likely demonstrating that these dysfunctional mitochondria are targeted for degradation.⁵⁰ In contrast, Moiseeva et al. found that mitochondrial mass and DNA content is increased in cells undergoing OIS.³⁹ These authors also observed a marked increase in mitochondrial superoxides, indicating a significant change in mitochondrial metabolism. Indeed, senescence can be directly induced by interference with normal mitochondrial metabolism using specific knockdown of the important mitochondrial protein Rieske iron sulfur protein (RISP) or the pharmacologic inhibitors rotenone and oligomycin.³⁹ Although studies have shown some conflicting results, it is clear that mitochondrial metabolism is markedly changed during OIS and may regulate senescence.

In addition to changes in mitochondria themselves, flux through the mitochondrial TCA cycle is increased in cells undergoing OIS. One study has demonstrated a significant increase in TCA cycle metabolites in OIS cells (Fig. 1).⁴² This is likely due to both an increase in the mitochondrial pyruvate gatekeeper enzyme PDH^{42,43} and an increase in enzymes in the TCA cycle such as malate dehydrogenase (MDH1/2) and succinate

dehydrogenase (SDHA).⁴³ Increased PDH activity results from decreased expression of the PDH suppressive pyruvate dehydrogenase kinase 1 (PDK1) and increased expression of the PDH activating pyruvate dehydrogenase phosphatase 2 (PDP2) (Fig. 1).^{42,51}

Oxidative phosphorylation is one of the most important biological processes of the mitochondrion.⁴⁷ A number of studies have shown that oxphos is increased during OIS, leading to an increase in oxygen consumption (Fig. 1).^{38,42,52} This is likely due to increased TCA cycle metabolites and enzyme expression^{42,43} in addition to increased expression of all complexes of the electron transport chain.⁴³ Increased oxygen consumption and oxphos complex enzyme expression should lead to an increase in ATP levels. However, a number of studies have shown a decrease in ATP levels in cells undergoing OIS.³⁹⁻⁴¹ This may be due to an increased usage of ATP by senescent cells for other highly energetic metabolic processes.

As discussed above, cancer cells show a marked decrease in TCA cycle and oxidative phosphorylation compared to normal cells. However, this is not due to mitochondrial defects in tumor cells,³⁵ but instead is most likely due to an increase in glucose uptake and glycolytic enzymes in addition to an increase in the PDH inhibitory PDK enzymes.^{34,44} This leads to hyperactivation of aerobic glycolysis and less pyruvate entering the TCA cycle. As discussed above, loss of p53 can suppress OIS,^{10,32} and p53 is known to be important for mitochondrial respiration and as a negative transcriptional regulator of aerobic glycolysis (Fig. 1). In part, this occurs through negative regulation of PDK2 (Fig. 1).⁵³ Loss of p53 thereby leads to an increase in PDK2, which in turn reduces the amount of pyruvate entering the mitochondria for the TCA cycle.⁴⁴ Additionally, loss of pRB, which can suppress OIS,¹² leads to decreased mitochondrial oxidative phosphorylation.^{30,54} This indicates that suppression of OIS through loss of p53 and/or pRb can switch metabolism from the mitochondria to a more Warburg effect type of phenotype.

Lipid metabolism is altered during OIS

Lipids are important for a number of cellular processes, including energy storage and signaling, and as components of cellular membranes.⁵⁵ Lipids can be grouped into 8 different categories including 2 lipids that will be discussed below: fatty acids and phospholipids. Importantly, fatty acids can be used as a major source of energy during a process called fatty acid oxidation (or β -oxidation) in which these lipids are catabolized into acetyl-CoA to be used in the TCA cycle. During β -oxidation, fatty acids are transported across the outer mitochondrial membrane by carnitine-palmitoyl transferase I (CPT1), which is rate-limiting for this process.⁵⁶ Although phospholipids are generally thought to be most important as a major component of cellular membranes, they can also be signaling messengers. Phospholipids are generated from fatty acids and can have different head groups; in particular, choline is the head group of phosphatidylcholine, which is the main constituent of cell membranes.⁵⁷ These phospholipids can be catabolized into free fatty acids to generate ATP under periods of cell stress.⁵⁵

Recent studies have shown a change in lipid metabolism in cells undergoing OIS. Quijano et al. recently demonstrated that senescent cells display an elevation in free fatty acid levels (Fig. 1).⁵² However, this is not due to an increase in lipid metabolism because these authors showed a decrease in acetyl-CoA carboxylase (ACC), the rate-limiting enzyme in lipid metabolism, in cells undergoing OIS. Instead, an increase in fatty acid oxidation was found to be the dominant factor in the increase in oxygen consumption in these cells. This may be another reason why senescent cells display an increase in oxygen consumption but no appreciable increase in ATP levels. The mechanism by which increased fatty acid levels are increased in OIS cells is unclear but may be due to promyelocytic leukemia (PML) activation of the fatty acid oxidation pathway through peroxisome proliferator-activated receptor (PPAR) signaling.⁵⁸ PML and PML nuclear bodies are known to play a major role during OIS.^{59,60} Increased levels of fatty acids and their subsequent degradation, leading to increased NADPH levels, may occur because of an increased need for antioxidants to combat the high levels of reactive oxygen species (ROS) observed during OIS.^{39,61} Interestingly, changes in fatty acid oxidation were shown to play a role in the senescence-associated secretory phenotype (SASP). Knockdown of CPT1, the rate-limiting enzyme in fatty acid oxidation, decreased the SASP during OIS.⁵² This suggests that fatty acid oxidation is necessary for SASP expression during OIS. Another recent study also indicates an alteration in lipid metabolism during OIS. Gey et al. found a specific decrease in phosphocholine (PC) and an increase in L- α -glycerophosphocholine (GPC), suggesting catabolism of phospholipids via phospholipase A1 and/or A2 and lysophospholipase.⁶² Indeed, another study showed alterations specifically in mitochondrial phospholipids,⁵⁰ which may play a role in the mitochondrial dysfunction observed during OIS. These studies suggest that the changes in lipid metabolism observed during OIS may play a specific role in the senescence phenotype, especially the SASP.

It has become apparent in recent years that lipid biogenesis is altered in cancer cells.⁴⁸ In contrast to cells that have undergone OIS, which display increased fatty acid catabolism, many cancer cells show a significant increase in *de novo* fatty acid synthesis.⁴⁸ This is likely due to increased expression of key enzymes in this pathway, including ATP-dependent citrate lyase (ACLY), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FASN).^{34,48} A number of the fatty acid pathway enzymes are regulated by AKT.⁶³ Interestingly, activation of AKT or suppression of PTEN in cells that have undergone OIS can overcome the cell growth arrest.^{64,65} Although AKT/PTEN is involved in a number of different pathways, these data suggest that overcoming OIS through upregulation of these pathways may be in part due to a change in lipid biogenesis.

Autophagy and lysosomal activity is increased during OIS

Autophagy is literally the process by which a cell eats itself.⁶⁶ Macroautophagy, which is the process discussed in this review, delivers cytoplasmic materials to an autolysosome via an autophagosome. Therefore, autophagy and the lysosomal compartment are intimately linked. Under nutrient-rich conditions, the

mTORC1 complex generally inhibits autophagy.⁶⁷ Under nutrient-poor conditions, mTORC1 is inhibited, leading to upregulation of a number of autophagy-related genes (Atg genes) and initiation of the phagophore. Autophagy is thought to be a cell survival mechanism and has been implicated in several human diseases including cancer.⁶⁶

A number of studies have indicated that autophagy is upregulated during OIS. LC-III, a marker of autophagy, and the number of autophagic vesicles are both significantly increased in cells undergoing OIS (Fig. 1).⁶⁸ Indeed, long-lived proteins are degraded at an increased rate in OIS cells compared to proliferating cells. Autophagy is likely increased during OIS as a result of repression of the mTORC1 and mTORC2 complexes through a negative feedback loop.⁶⁹ Furthermore, induction of autophagy was found to be necessary for OIS as knockdown of key autophagic proteins such as ATG5 or ATG7 bypassed senescence induced by RAS, BRAF, or the KSHV v-cyclin A protein.^{68,70-73} It is known that senescent cells display an increase in protein content.^{43,74} Therefore, during senescence, autophagy is probably activated to increase the amino acid content in order to cope with the higher rate of protein translation.⁷⁵

Similar to the increase in autophagy, a number of studies have observed an increase in lysosomal activity in cells undergoing OIS. This is likely because autophagy and lysosomes go hand-in-hand to quickly degrade proteins and other biomolecules within the cell. Young et al. observed a significant increase in lysosomal genes in cells undergoing OIS.⁶⁸ These authors also observed an increased lysosomal compartment using LysoTracker and electron microscopy (Fig. 1). The increase in lysosomal gene expression directly correlates with the timing of the increase in autophagy genes, indicating coordinated upregulation of both cellular processes during OIS. A more recent study further identified the mechanism of upregulation of lysosomal genes. Urbanelli et al. demonstrated a significant increase in the transcription factor TFEB that leads to upregulation of HEXA and HEXB, which encode lysosomal glycohydrolase β -hexosaminidase.⁷⁶ Taken together, these studies indicate an increase in both autophagy and lysosomal activity in cells undergoing OIS.

Subversion of the autophagic process can overcome OIS⁶⁸ and potentially lead to transformation of cells. Indeed, autophagy is thought to be a tumor suppressive mechanism.⁶⁶ Autophagy is negatively regulated by mTOR,⁷⁷ which is induced during AKT signaling.⁷⁸ As discussed above, increased AKT signaling through activated AKT or inactivated PTEN can overcome the OIS-associated cell growth arrest.^{64,65} In addition to the role of this pathway in other metabolic processes, this may also be due to inhibition of autophagy. Autophagy is also positively regulated by pRb (Fig. 1),⁷⁹ and loss of pRb can suppress OIS,⁶⁸ which may in part be due to a decrease in autophagy. Together, these studies suggest that overcoming OIS through suppression of autophagy could lead to transformation and tumorigenesis.

Targeting the metabolome as a pro-senescence cancer therapeutic strategy

Senescence is now thought to be a viable outcome for cancer therapy.⁸⁰ Additionally, targeting the altered metabolome in

cancer cells has attracted great interest in recent years.^{47,81,82} Numerous compounds that have been developed to target different metabolic pathways are reviewed elsewhere.^{47,81,82} Here, we will review recent studies that have shown that shifting metabolism toward that seen during OIS could be a viable strategy to induce cancer cell senescence and/or regression of tumors.

Targeting nucleotide metabolism as a pro-senescence cancer therapy

Cells that have undergone OIS show a marked decrease in dNTP levels and the rate-limiting enzyme RRM2.^{9,22} Overcoming the decrease in nucleotide metabolism can lead to re-entry of fully senescent cells into the cell cycle.⁹ To sustain a high level of proliferation, cancer cells require elevated levels of dNTPs.²⁴ It is possible to exploit this need by targeting this pathway to induce cancer cell senescence. A number of inhibitors of the nucleotide metabolic pathway are currently being used in the clinic.^{11,81} Studies from our laboratory have shown that inhibition of nucleotide metabolism through specific knockdown or inhibition of RRM2 using 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP)⁸³ can induce senescence in multiple cancer cell models with oncogenic signaling activation.^{9,84} Notably, senescence induced by RRM2 inhibition is independent of p53 and p16 and correlates with the DNA damage response.^{9,84} This supports the premise that inhibition of this pathway in cancer cells is a viable pro-senescence therapeutic strategy. The dNTP metabolic pathway has been exploited for cancer treatment for decades. New insights suggest that it remains an important target for the development of novel cancer therapeutic strategies using senescence as a primary tumor suppression mechanism.

Targeting glucose metabolism as a pro-senescence cancer therapy

During OIS, cells display a significant shift in glucose metabolism toward the mitochondrial TCA and oxidative phosphorylation pathways and away from the Warburg effect and fermentation (discussed above). Increased aerobic glycolysis and fermentation are hallmarks of cancer cells.²⁴ Therefore, suppression of OIS may alter glucose metabolism to a more Warburg effect-like phenotype. Because aerobic glycolysis is not observed in normal cells, targeting this pathway has been of major interest in recent years; indeed, a number of agents are being developed to target this pathway for cancer therapy.^{81,82} For example, a small molecule of the GLUT1 transporter (WZB117) induces senescence and inhibits tumor growth *in vivo*.⁸⁵ Although a number of other inhibitors of glycolysis have been developed, most have not moved past Phase I/II clinical trials and their development has been discontinued.⁸² This is most often due to unacceptable toxicities.⁸¹ Future inhibitors will need to be more specific to limit off-target effects. Nevertheless, these data support the hypothesis that shifting metabolism away from the Warburg effect is a viable pro-senescence cancer therapeutic strategy.

Targeting mitochondrial metabolism

OIS cells display a significant increase in TCA cycle intermediates and oxidative phosphorylation (discussed above).

Although cancer cells have functional mitochondria,³⁵ most of their ATP is produced through glycolysis.³⁶ Therefore, drugs that shift metabolism toward the mitochondria may induce senescence of cancer cells. For instance, upregulation of PDH by knockdown of PDK1, which would shunt pyruvate into the TCA cycle,⁴⁴ can cause regression of melanoma tumors with oncogenic BRAF mutations.⁴² In addition, knockdown of malic enzymes (ME1/2) can induce senescence of tumor cells.⁸⁶ ME1/2 metabolize malate into pyruvate, thereby producing NADPH, which is necessary for a number of metabolic processes including nucleic acid synthesis.⁴⁴ Thus, targeting the mitochondrial pathways can exert an effect on other metabolic pathways known to play a role in senescence.

Concluding Remarks

In recent years, it has becoming increasingly clear that OIS is accompanied by a significant alteration in cellular metabolism. Given that OIS is a tumor suppressor mechanism,^{4,5} the changes in cellular metabolism are mostly opposite to those observed during tumorigenesis. For example, during OIS glucose metabolites are mainly shunted toward the TCA cycle and oxidative phosphorylation. This is in contrast to tumor metabolism, which shunts glucose either to fermentation to produce lactate or the PPP, thereby increasing the level of nucleotides and other necessary biomolecules for proliferation. This indicates that these pathways play a role in the tumor suppressive phenotype of OIS. Understanding these alterations in the metabolome may give researchers further insight into how cells are able to circumvent the OIS phenotype to become cancerous. Indeed, a number of key senescence effectors such as p53 and pRb also play major roles in metabolism.^{30,34,44,46,53,54,79} This underscores the importance of further understanding these pathways to potentially prevent the bypass of OIS. Additionally, this knowledge may allow us to exploit cancer cells' metabolic weaknesses as a therapeutic strategy. Interestingly, a recent study found that exploiting metabolic changes during therapy-induced senescence of cancer cells could induce a synthetic lethality.³⁸ This suggests that these changes can be targeted in cancer patients to improve cancer therapy. Further studies are therefore warranted to fully understand the metabolic alterations accompanying OIS and how these are changed when cells overcome the OIS-associated cell cycle exit.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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