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Cellular metabolic stress: Considering how cells respond to nutrient excess

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Abstract

Nutrient stress is generally considered from the standpoint of how cells detect and respond to an insufficient supply of nutrients to meet their bioenergetic needs. However, cells also experience stress as a result of nutrient excess, during which reactive oxygen species (ROS) production exceeds that required for normal physiological responses. This may occur due to oncogene activation or chronic exposure to growth factors combined with high levels of nutrients. As a result, multiple mechanisms have evolved to allow cells to detect and adapt to elevated levels of intracellular metabolites, including: promotion of signaling and proliferation by ROS, amino acid-dependent mTOR activation, and regulation of signaling and transcription through metabolite-sensitive protein modifications. We discuss how each of these responses can contribute to the development and/or progression of cancer under conditions of cellular nutrient excess and their potential roles in linking chronic organismal over-nutrition (obesity) with cancer.

Introduction

Core metabolic pathways have been generally well conserved among eukaryotes; organisms from yeast to mammals make use of both glycolytic and mitochondrial metabolism depending on extracellular conditions and cues, cellular needs, and stage of metabolic or circadian cycle (DeBerardinis et al., 2008; Sahar and Sassone-Corsi, 2009; Tu et al., 2005) Despite these similarities, fundamental differences exist between unicellular and multicellular organisms in the acquisition of nutrients and control of metabolism. While unicellular organisms must deal with the potentially large fluctuations in nutrient availability in the extracellular environment, cells within larger multicellular organisms have access to a relatively stable supply of nutrients from the bloodstream. While some cell types such as liver, muscle, and fat cells have the capacity to store excess carbon in the form of glycogen or lipid, most cells are unable to assimilate excess nutrients in the absence of engaging in cell growth and/or proliferation. Nutrient uptake in metazoan cells is controlled primarily by growth factor signaling. Thus too much or too little growth factor signaling-induced nutrient uptake can profoundly affect cellular bioenergetic fitness. A major readout of growth factor-regulated nutrient uptake is the level of reactive oxygen species (ROS) produced by mitochondria. In this review, we discuss how high levels of nutrient metabolism can stress the cell, the mechanisms used by the cell to detect and respond to elevated intracellular metabolite levels, and the contribution of cellular and organismal nutrient excess to cancer.

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Nutrient excess and cellular stress

ROS are produced at a low level by the electron transport chain as a normal part of cellular metabolism and play a physiologically important role in the regulation of cell signaling, proliferation, and differentiation. ROS production can rise, however, with changes in oxidative mitochondrial metabolism, potentially causing damage to cellular components and cell death (Hamanaka and Chandel, 2010; Trachootham et al., 2009; Veal et al., 2007). Cells experience stress as a result of "nutrient excess" when ROS production exceeds that needed for normal physiological responses (Figure 1). It is not coincidental that diseases characterized by altered cellular metabolism, such as cancer and diabetes, are also characterized by elevated ROS levels that may contribute to disease pathogenesis (Brandon et al., 2006; Halliwell, 2007; Nathan, 2008; Roberts and Sindhu, 2009; Trachootham et al., 2009; Wallace, 2005).

How do changes in metabolism impact mitochondrial ROS production? The tricarboxylic acid (TCA) cycle oxidizes nutrients, and the resulting electrons are transferred to nicotinamide adenine dinucleotide (NAD+) and flavin adenine dinucleotide (FAD) to produce NADH and $FADH_2$. These electrons are donated to the electron transport chain (ETC) at complexes I and II, respectively. Electrons are shuttled from complexes I and II to complex III via ubiquinone and from complex III to complex IV via cytochrome c. Two electrons are finally donated to molecular oxygen (½ O_2), generating H_2O. Incomplete, one-electron reduction of oxygen can also occur at complexes I, II, and III, producing superoxide (O_2^{-}•). Superoxide production increases with the concentration of oxygen and electron donors (Turrens, 2003). Hence, rising levels of NADH/NAD+ is a major factor leading to increased mitochondrial ROS production (Murphy, 2009). When breakdown of metabolites in the TCA cycle exceeds the capacity of the ETC to assimilate the resulting electrons, ROS production increases. In addition, stalling of the ETC, due to mutated oxidative phosphorylation (OXPHOS) genes can cause build-up of electrons and increased propensity to produce superoxide (Brandon et al., 2006; Wallace, 2005). Since the mitochondrial genome, which encodes several OXPHOS genes, is exposed to elevated levels of ROS due to its proximity to the ETC, conditions that favor increased ROS production such as excessive metabolite-dependent conversion of NAD+ to NADH could potentially lead to a feed-forward loop of increased ROS production and mitochondrial DNA (mtDNA) mutation (Trachootham et al., 2009).

While superoxide mediates its effects within a short range of its production, it can also be converted by superoxide dismutases (SODs) into hydrogen peroxide (H_2O_2), which is more stable and can diffuse throughout the cell. H_2O_2 is a known regulator of signaling through oxidation of redox sensitive proteins, such as protein tyrosine phosphatases (PTPs) (Meng et al., 2002); hence, electrons generated by excess mitochondrial metabolism can be used to regulate intracellular signaling through production of ROS. H_2O_2 can also be converted into the highly reactive hydroxyl radical (OH•) in the presence of reduced transition metals, via the Fenton reaction; in this manner, ROS production in the mitochondria can lead to damage to macromolecules throughout the cell, including nuclear DNA, membrane lipids, and proteins.

Mitochondrial ROS production also rises under hypoxic conditions, and these ROS are involved in activation of hypoxia inducible factors (HIFs), which mediate a transcriptional response that promotes adaptation to hypoxia (Brunelle et al., 2005; Guzy et al., 2005; Guzy and Schumacker, 2006; Mansfield et al., 2005). ROS production under hypoxic conditions occurs primarily at complex III, although the precise mechanisms involved are not yet clear (Bell et al., 2007; Guzy and Schumacker, 2006). Other mitochondrial sources of ROS may also be important in addition to that produced from the ETC. For example, during nutrient

deprivation, the mitochondrial enzyme proline oxidase (POX) is induced and can augment TCA cycle function by converting proline into pyrroline-5-carboxylate, which can then be converted into the TCA cycle metabolite α-ketoglutarate (Phang et al., 2010). The POX reaction is also a source of superoxide, and POX-dependent ROS production has been shown to regulate signaling and promote apoptosis under stress conditions (Phang et al., 2010). Tumor cells may encounter both types of ROS-inducing nutrient stress: 1) oncogene-driven increases in nutrient metabolism and 2) nutrient-deprivation and hypoxia as tumor growth outpaces angiogenesis.

In addition to rate of production, the cumulative effects of ROS are contingent on antioxidant activity. As discussed, superoxide accumulation is limited by the presence of superoxide dismutases (SODs), which rapidly convert superoxide into hydrogen peroxide (H_2O_2) and oxygen. Hydrogen peroxide can be reduced to water by enzymes such as catalase, glutathione peroxidases, and peroxiredoxins distributed throughout the cell. Under normal conditions, ROS production and scavenging are balanced by the cell to maintain ROS levels within a low physiological range. In contrast, many cancer cells have altered antioxidant defenses. The tumor suppressor p53, for example, plays a critical role in cellular antioxidant defenses, and its downregulation is associated with reduced levels of antioxidant production and increased DNA oxidation and mutation (Sablina et al., 2005). Akt activation, which occurs commonly in cancer as a result of mutations in upstream signaling components, is also associated with higher mitochondrial oxygen consumption and ROS generation, coupled with reduced ROS scavenging, due to Akt-dependent inhibition of FoxO transcription factors (Noguiera et al., 2008). FoxO transcription factors, which are themselves tumor suppressors, regulate the expression of multiple ROS detoxifying enzymes, among other effects (Dansen and Burgering, 2008). While increased ROS production contributes to tumorigenesis, ROS levels must be restrained even in cancer cells if they are to avoid the damaging effects of ROS on the integrity of vital intracellular macromolecules. Therapeutic augmentation of ROS levels has the potential to be selectively toxic to cancer cells; both Ras transformation and Akt activation sensitize cells to phenethyl isothiocyanate (PEITC), a substance which inactivates the glutathione antioxidant system (Nogueira et al., 2008; Trachootham et al., 2006).

The point at which ROS production crosses the line from physiological to pathological is often not clear. Sensitivity to ROS varies among cell types, and location of production and species of ROS can lead to different biological outcomes. This is exemplified by the regulation of insulin sensitivity by ROS. Activation of NADPH oxidases at the plasma membrane upon insulin binding to its receptor promotes insulin action, via H_2O_2-dependent inactivation of PTPs that inhibit insulin signaling (Goldstein et al., 2005). This temporal and highly regulated ROS generation promotes sensitivity to insulin. In contrast, chronic ROS production has been demonstrated to cause insulin resistance, and increased ROS production has been implicated in linking obesity with diabetes (Houstis et al., 2006; Roberts and Sindhu, 2009). Indeed, eating a high fat diet (HFD) has been shown to increase the ROS-emitting potential of mitochondria in both rats and humans (Anderson et al., 2009). Reducing this ROS production through antioxidant treatment or through over-expression of mitochondria-targeted catalase in muscle improves insulin sensitivity in obese rodents (Anderson et al., 2009; Houstis et al., 2006). Interestingly, even in the context of HFD, ROS production can also promote insulin sensitivity; mice lacking the H_2O_2 scavenger glutathione peroxidase 1 are protected from HFD-induced insulin resistance due to increased oxidation of PTEN, a redox-sensitive PTP, thus enabling increased signaling downstream of the insulin receptor (Loh et al., 2009). These findings highlight the complexity of ROS biology and point to the importance of location and duration of ROS production, as well as species of ROS in determining its cellular impact.

Sensing nutrient excess: Cellular metabolism can promote cancer development

Nutrient uptake in mammalian cells is controlled by growth factor signaling, allowing nutrient consumption to increase transiently when growth factors bind to their cell surface receptors and activate downstream signaling cascades (Figure 2). Prolonged high levels of nutrient metabolism can occur due either to 1) a chronic combination of excess nutrients and insulin/growth factors or 2) to oncogenic activation of signaling pathways.

Nutrient availability is sensed at multiple levels by the cell. AMP-activated protein kinase (AMPK) inhibits growth and proliferation in response to ATP depletion and/or AMP accumulation. Mammalian target of rapamycin (mTOR) is activated by growth factor signaling in order to promote protein synthesis and cell growth, in a manner dependent on intracellular amino acid levels. These kinases are sensitive to nutrient deficiency and instruct cells as to whether available nutrients are sufficient to permit growth. In addition, both mitochondrial and endoplasmic reticulum (ER) stress responses can regulate or induce adaptation to the ROS production initiated by nutrient excess. Finally, mounting evidence suggests that metabolically sensitive protein modifications, such as acetylation, O-GlcNAcylation, and N-glycosylation, may each play key roles in cellular response to nutrient excess and are likely to be relevant in metabolic diseases and cancer (Haigis and Sinclair, 2010; Lau and Dennis, 2008; Schwer and Verdin, 2008; Slawson et al., 2010) (Figure 3).

Excess Mitochondria-Produced ROS and Disease

Mitochondrial ROS production increases with oncogene-induced metabolic stress, and substantial evidence indicates that elevated ROS levels can promote tumorigenesis (Halliwell, 2007; Wallace, 2005). While cancer cells are often thought of as relying exclusively on glycolytic metabolism, it is now clear that mitochondrial metabolism is also essential, particularly in supporting cellular biosynthesis (DeBerardinis et al., 2008). In proliferating cells utilizing aerobic glycolysis, glutamine plays a key role in supplying substrate to the TCA cycle (DeBerardinis et al., 2007). Glutamine uptake and mitochondrial metabolism are both regulated by pro-growth signaling pathways and oncoproteins. For example, Myc stimulates glutamine uptake, glutaminolysis, and mitochondrial biogenesis (Gao et al., 2009; Li et al., 2005; Wise et al., 2008). mTOR also plays a key role in promoting increased mitochondrial metabolism and oxygen consumption and can stimulate net uptake of amino acids (Edinger and Thompson, 2002; Schieke et al., 2006). As we will discuss, increased production of ROS, largely from the mitochondria, may promote cancer by increasing DNA mutation, by regulating signaling and transcription, and by promoting inflammation.

Direct damage to and mutation of DNA due to elevated ROS production can contribute to cancer development. A number of mouse models containing reduced levels of antioxidants exhibit increased cancer development or progression, associated with increased oxidative damage to DNA and other macromolecules (Chu et al., 2004; Elchuri et al., 2005; Frohlich et al., 2008; Neumann et al., 2003; Sablina et al., 2005). ROS-induced damage to DNA and DNA repair enzymes can induce genomic instability, and mutation of nuclear encoded genes such as *TP53* promotes carcinogenesis (Colotta et al., 2009; Hussain et al., 2003). Mutation of mtDNA can also increase ROS production and promote cancer progression, as demonstrated in experiments using cybrid (cytoplasmic hybrid) technology, which allows the generation of cells that retain their own nuclear genome but contain mtDNA from another source (Brandon et al., 2006). Cybrid PC-3 prostate cancer cells were generated containing either wild type cytochrome oxidase subunit I (COI) or COI containing a

mutation found in multiple independent prostate tumors. Remarkably, when these cells were grown in nude mice, the mutant COI-containing tumors grew to roughly 7 times the size of the tumors expressing wild type COI (Petros et al., 2005). Furthermore, similar experiments in which cybrids were generated containing mutations in NADH dehydrogenase subunit 6 (ND6), causing deficient complex I activity and elevated ROS production, led to increased metastasis in poorly metastatic recipient cells (Ishikawa et al., 2008). Thus, mutation of either mtDNA or nuclear DNA in the presence of ROS can contribute to cancer development.

Signaling and transcriptional changes in response to elevated ROS are also relevant to cancer development. ROS-dependent inactivation of PTPs has been reported to be increased in cancer cells and may also enhance pro-growth signaling and contribute to cancer in this way (Lou et al., 2008; Wu, 2006). Regulation of signaling by ROS has consequences for cancer cell proliferation; for example, mitochondrial ROS production was shown to be critical for anchorage-independent growth driven by oncogenic Kras, likely through the regulation of ERK1/2 signaling (Weinberg et al., 2010). Additionally, hypoxia-induced ROS is important for activation of hypoxia inducible factors (HIFs), which can regulate tumorigenesis through transcriptional induction of genes involved in angiogenesis, invasion and metastasis, dedifferentiation, and glycolytic metabolism, as well as through interactions with proteins such as Myc and p53 (reviewed in (Majmundar, 2010)). ROS-induced stabilization of HIF-1α was also shown to be critical for Myc-dependent tumorigenesis and an important target of antioxidants in inhibiting tumor growth (Gao et al., 2007).

Activation of inflammatory pathways is another key link between ROS and cancer. Much evidence indicates that cancer is frequently associated with a state of chronic inflammation, and that inflammation plays a key role in every stage of cancer development and progression (Coussens and Werb, 2002; Grivennikov et al., 2010; Karin and Greten, 2005; Mantovani et al., 2008). ROS are implicated in activating the transcription factors NF-κB and AP-1, mainly through activation of the upstream kinases that control their activation, inhibitor of kappa B kinase(IKK) and c-Jun N-terminal kinase (JNK) (Gloire et al., 2006; Kamata et al., 2005; Sen and Packer, 1996). Inflammation in cancer involves a close interplay between tumor-associated immune cells and the tumor cells themselves. Activation of NF-κB and AP-1 in immune cells induces production of inflammatory cytokines such as TNFα and IL-6. Inflammatory cytokine signaling in tumor cells promotes further increases in ROS production, stimulation of proliferation through Stat3, and promotion of cell survival and further recruitment of immune cells through transcriptional targets of NF-κB. The importance of the IKK-NF-κB pathway, TNFα, IL-6, and Stat3 in tumorigenesis has been demonstrated in multiple murine cancer models (Grivennikov et al., 2010).

Nutrient adaptation and the ER stress response

Cellular redox homeostasis and inflammatory status are also regulated by the unfolded protein response (UPR) in response to ER stress caused by factors such as nutrient deprivation or nutrient excess. The UPR is mediated primarily by three ER-membrane associated proteins PERK, IRE1, and ATF6, which together act to relieve ER stress by reducing protein translation and assisting protein folding and degradation of misfolded proteins (Buchberger, 2010; Kaufman et al., 2002). Inflammatory pathways crosstalk with the UPR through multiple mechanisms; each of the three arms of the UPR has been implicated in activation of NF-κB, and JNK can be activated through IRE1α (reviewed in (Hotamisligil, 2010)). Adaptation to oxidative stress can in part be regulated by the UPR through PERK-dependent activation of Nrf2, a transcription factor that induces antioxidant gene expression (Cullinan and Diehl, 2006). Markers of ER stress are altered in cancer and substantial evidence implicates the involvement of ER stress in cancer (Healy et al., 2009; Lee, 2007; Moenner et al., 2007). Evidence that regulation of redox homeostasis by the

PERK-Nrf2 arm of the UPR may impact cancer development has recently been provided. Suppression of PERK in cancer cells was associated with increased ROS levels and activation of a DNA damage checkpoint, and in MMTV-*Neu*-driven mammary tumors, deletion of PERK inhibited tumor growth (Bobrovnikova-Marjon et al., 2010). Interestingly, mammary gland-specific PERK deletion also led to an increase in spontaneous tumors, likely due to increased genomic instability (Bobrovnikova-Marjon et al., 2010).

Acetylation as a Nutrient-Regulated Protein Modification

Protein modifications such as acetylation and glycosylation can be regulated in a metabolically responsive manner and might also contribute to adaptation to metabolic stress (Figure 3). In mammalian cells, nucleo-cytosolic acetyl-CoA is generated by two enzymes, acetyl-CoA synthetase 1 (AceCS1), which produces acetyl-CoA from acetate, and ACL, which generates acetyl-CoA through the cleavage of citrate. Citrate is produced in the TCA cycle and exported by the tricarboxylate transporter from the mitochondria into the cytosol, where it is accessible to ACL. The regulation of histone acetylation is sensitive to nutrient availability, in a manner dependent on ACL (Wellen et al., 2009). Moreover, ACL-dependent acetylation may be sensitive to differences in nutrient metabolism at the high end of the spectrum; higher levels of histone acetylation were observed when adipocytes were differentiated in 25 mM as compared to 4 mM glucose, correlating with specific changes in gene expression (Wellen et al., 2009). Recent studies have demonstrated that nutrient-dependent acetylation extends beyond histones; lysine acetylation of various metabolic enzymes is also metabolically responsive (Wang et al., 2010a; Zhao et al., 2010). Proteomic analyses have revealed that thousands of proteins in mammalian cells are acetylated (Choudhary et al., 2009; Zhao et al., 2010), and the list of proteins acetylated in a nutrient-responsive manner will undoubtedly continue to grow. Interestingly, removal of acetyl groups from proteins is also metabolically sensitive, through the action of NAD+-dependent sirtuin protein deacetylatases. NAD+ is consumed during glycolytic and oxidative metabolism, and calorie-restricted conditions promote activation of sirtuins (Haigis and Sinclair, 2010; Schwer and Verdin, 2008). The combined action of deacetylation by sirtuins under low nutrient conditions and ACL-dependent acetylation under nutrient-replete conditions offers the possibility for protein acetylation levels to participate in coupling metabolism to protein function over a wide range of nutritional conditions.

Is metabolic regulation of acetylation relevant to cancer? Certainly more work is needed. RNAi-mediated suppression of ACL has been demonstrated to inhibit tumor growth (Hatzivassiliou et al., 2005). While this is due in large part to the critical role of ACL in *de novo* lipogenesis, which is important for membrane synthesis in proliferating cells, it is possible that effects on acetylation could also play a more direct role in carcinogenesis. Suppression of ACL promotes differentiation in cancer cells and myoblasts (Bracha et al., 2010; Hatzivassiliou et al., 2005), and it is an intriguing possibility that changes in acetylation of histones or other proteins might be part of the mechanism through which ACL can regulate differentiation. Evidence for this possibility was suggested in the study in myoblasts; treatment with the histone deacetylase inhibitor trichostatin A could reverse ACL suppression-dependent differentiation (Bracha et al., 2010).

Glycosylation as a Nutrient-Sensitive Modification

Protein N-linked glycosylation and O-linked N-acetylglucosamine (O-GlcNAc) modification can also each be regulated in a nutrient-responsive manner, at least in part through the flux of glucose into the hexosamine biosynthetic pathway. The hexosamine pathway is a relatively minor branch of glucose metabolism, representing the fate of roughly 3–5% of glucose entering the cell (Marshall et al., 1991). In addition to glucose, glutamine and acetyl-CoA are necessary for pathway activity, suggesting that the hexosamine pathway

is highly attuned to cellular metabolism. The hexosamine pathway diverges from glycolysis at fructose-6-phosphate, where glutamine-fructose-6-phosphate amidotransferase (GFAT) transaminates fructose-6-phosphate to produce glucosamine-6-phosphate. The end product of the pathway is UDP-GlcNAc, which is critical for both nucleocytoplasmic O-GlcNAc protein modification and N-linked glycosylation in the ER and Golgi (Dennis et al., 2009; Love and Hanover, 2005).

N-linked glycosylation of proteins occurs cotranslationally in the ER, and further remodeling of N-glycans takes place in the Golgi apparatus (Kornfeld and Kornfeld, 1985). UDP-GlcNAc is used for production of the lipid linked oligosaccharide (LLO) that is transferred onto asparagines residues of nascent polypeptides in order to initiate N-glycosylation in the ER (Kornfeld and Kornfeld, 1985). UDP-GlcNAc is also transported into the Golgi, where it is used by 4 N-acetylglucosaminyltransferases (MGAT1, 2, 4, and 5) to sequentially modify N-glycan branching (Dennis et al., 2009). These enzymes possess differential affinities for UDP-GlcNAc and the glycoprotein acceptors, such that the activities of MGAT4 and MGAT5 depend on the concentration of UDP-GlcNAc in the Golgi (reviewed in (Dennis et al., 2009).

Importantly, MGAT5 activity and UDP-GlcNAc availability have been shown to modulate the surface expression of various growth factor receptors such as the EGFR, directly regulating growth factor-dependent signaling (Lau et al., 2007; Partridge et al., 2004). In addition, MGAT4-dependent regulation of glycosylation of the glucose transporter Glut2 has been shown to promote its retention at the cell surface of pancreatic β cells. In the absence of MGAT4, Glut2 is found primarily in endosomes and lysosomes rather than at the cell surface, and glucose-stimulated insulin secretion is markedly inhibited (Ohtsubo et al., 2005).

O-linked GlcNAc modification occurs in a reversible manner on serine and threonine residues of a wide range of intracellular proteins, from signaling proteins and transcription factors to metabolic enzymes, and accumulating evidence indicates that O-GlcNAc is key to many cellular functions (Love and Hanover, 2005; Zachara and Hart, 2004). Proteins are modified by O-GlcNAc through the action of a single enzyme, O-GlcNAc transferase (OGT). Efforts to generate OGT null mice revealed that this protein is necessary for viability of embryonic stem cells, and conditional mutagenesis of OGT in mice demonstrated that O-GlcNAc is critical for the viability and/or function all cell types examined (O'Donnell et al., 2004; Shafi et al., 2000). The ability of increased hexosamine pathway activation to mediate insulin resistance has been known for nearly two decades, and subsequent characterization has identified a key role for the O-GlcNAc modification in mediating these effects (Marshall, 2006; Teo et al., 2010). OGT is recruited to the plasma membrane in response to insulin, where it can bind phosphatidylinositide-(3,4,5)-triphosphate (PIP_3), presumably in order to place OGT in proximity to proteins in the insulin signaling cascade (Yang et al., 2008). OGT has also been reported to associate with PDK-1 and the insulin receptor (IR), which phosphorylates and activates OGT (Whelan et al., 2010; Whelan et al., 2008). O-GlcNAc modification has been demonstrated on a number of proteins in the insulin signaling cascade, and at least one mechanism that appears to be involved in O-GlcNAc-mediated inhibition of insulin action is the impairment of IRS-1 tyrosine phosphorylation at a PI3K p85 binding site, resulting in reduced interaction between IRS-1 and PI3K (Whelan et al., 2010).

It is anticipated that hexosamine pathway flux would be elevated in cancer cells exhibiting increased glucose uptake, and indeed, evidence has been emerging that hexosamine pathway activity increases under conditions of oncogene activation and likely contributes to tumorigenesis. Recent studies have shown that O-GlcNAcylation increases upon Myc

activation and is elevated in breast cancer and leukemia cells (Caldwell et al., 2010; Gu et al., 2010; Morrish et al., 2009; Shi et al., 2010). In two xenograft breast cancer models, RNAi-mediated suppression of OGT led to either an almost complete suppression of tumor growth or reduced metastasis, despite equal growth of the primary tumor (Caldwell et al., 2010; Gu et al., 2010). These different phenotypes may reflect differences between the breast cancer cell lines or perhaps degree of OGT suppression. Given that OGT is known to modify a number of tumor suppressors and oncoproteins, including p53 and c-Myc, and that O-GlcNAcylation is critical for proper cell cycle control and cytokinesis, it appears likely that O-GlcNAcylation will prove to be an important player during tumorigenesis (Caldwell et al., 2010; Chou et al., 1995; Slawson et al., 2005; Wang et al., 2010b; Yang et al., 2006).

Additionally, UDP-GlcNAc may become available at increased levels in the Golgi in cancer cells, in order to regulate N-glycan modification. Such changes would have the potential to be functionally significant to the cancer cell. For example, tumor growth in mouse models of cancer driven either by PTEN heterozygosity or by polyomavirus middle T (PyMT) transgenic expression was significantly delayed in the absence of MGAT5 (Cheung and Dennis, 2007; Granovsky et al., 2000). Studies in mammary epithelial tumor cells from PyMT transgenic *Mgat5*−/− and *Mgat5*+/+ mice showed that the absence of MGAT5 resulted in impaired response to multiple growth factors, which could be rescued by re-expression of *Mgat5* (Partridge et al., 2004). The β1,6GlcNAc –branched N-glycans produced by MGAT5 interact with galectins at the cell surface and can oppose glycoprotein endocytosis; both EGFR and TGFβR were found at lower levels at the cell surface of PyMT *Mgat5*−/− than PyMT *Mgat5*+/+ cells (Partridge et al., 2004). Since whole body metabolic homeostasis and cell surface expression of glucose transporters such as Glut2 can be regulated at the level of N-glycan branching (Ohtsubo et al., 2005, Cheung et al., 2007), it is possible that metabolic fluxes in cancer cells may also regulate nutrient transporter surface expression and cellular acquisition of nutrients. Clearly, more research is needed to understand how metabolic regulation of glycosylation may contribute to cancer.

Thus, nutrient excess affects multiple mechanisms that couple metabolism to pathways that modulate cell growth and proliferation (Figure 2). The mitochondrial and ER responses to high levels of nutrients can contribute to cancer through ROS-mediated mutagenesis of DNA, regulation of signaling, and activation of inflammatory pathways. Metabolically sensitive protein modifications such as acetylation and glycosylation are also emerging as key players in regulating cell function in accordance with intracellular nutrient availability. Significant research effort will be required to understand the complex roles that these modifications are playing.

Obesity contributes to cancer development

As we have discussed in this article, changes in cellular metabolism contribute to the development and progression of cancer in a number of different ways. Are all of these metabolic changes mediated exclusively through cell autonomous mechanisms such as oncogenic mutations, or can over-nutrition at an organismal level also contribute to cancer development? Epidemiological evidence clearly indicates that it can (Calle and Kaaks, 2004). Obesity is associated with increased risk for several types of cancer, with percentage of cases attributable to overweight and obesity in the United States and Europe estimated at over 20% for several types of cancer and between 40–60% for both endometrial and oesophageal cancers (Calle and Kaaks, 2004). Several mechanisms have been proposed to explain the link between metabolic disease and cancer, particularly hyperinsulinemia and chronic low-grade inflammation. As insulin resistance develops in obese individuals, insulin secretion from pancreatic beta cells increases in compensation, causing hyperinsulinemia. High levels of insulin itself might promote tumorigenesis, though hyperinsulinemia can also

lead to increased production of insulin-like growth factor I (IGF-I), as well as suppression of IGF binding proteins 1 and 2, which bind to IGF, inhibiting its bioavailability (Gallagher and Leroith, 2010; Renehan et al., 2006). High circulating levels of free IGF-I can stimulate PI3K-Akt signaling, thus promoting increased glucose metabolism, increased mitochondrial ROS production, and cell growth and survival. Much data indicates that IGF-I can promote tumorigenesis (Gallagher and Leroith, 2010), and a recent study using a mouse model of non-obese insulin resistance has demonstrated that hyperinsulinemia can enhance IR/IGF-IR and Akt phosphorylation in mammary tissue and promote mammary tumor growth (Novosyadlyy et al., 2010). Notably, calorie restriction, which reduces circulating levels of insulin and IGF-1, was shown to impair tumor growth only from cells that were responsive to insulin and IGF-1 and lacked mutations that resulted in activation of the PI3K/Akt pathway (Kalaany and Sabatini, 2009). The relationship between insulin, IGF, and obesity is complex, however. While insulin levels show a positive linear correlation with both body mass index (BMI) and free IGF-I, non-linear relationships exist between BMI and either total or free IGF-I, so the degree to which this mechanism contributes to obesity-linked cancer remains to be completely defined (Renehan et al., 2006).

Obesity is also associated with a state of chronic low-grade inflammation, likely due to changes wrought by nutrient excess, such as mitochondrial ROS production and ER stress (Wellen and Hotamisligil, 2005). It is now well-established that obesity-linked inflammation is a key mediator of insulin resistance (Hotamisligil, 2006; Shoelson et al., 2006). Given the clear role that chronic inflammation plays in cancer development, it stands to reason that obesity-linked inflammation may also promote cancer.

Recent studies have provided genetic evidence for the involvement of obesity-induced inflammation in liver and pancreatic cancer. Both high fat diet (HFD)-induced obesity and genetic obesity were shown to promote hepatocellular carcinoma development following injection of the pro-carcinogen diethylnitrosamine (DEN) into mice (Park et al., 2010). Obese mice exhibited increased levels of inflammatory cytokines in liver and serum, and mice lacking either interleukin-6 (*IL-6*$^{-/-}$) or tumor necrosis factor signaling (*TNFR1*$^{-/-}$) were protected from the tumor-promoting effects of HFD (Park et al., 2010). Similarly, HFD was also shown to promote K-ras-induced pancreatic cancer in mice, in a manner dependent on TNF receptor signaling (Khasawneh et al., 2009). Interestingly, these mice do not exhibit hyperinsulinemia on HFD, suggesting that obesity-linked inflammation can promote tumorigenesis independent of effects on insulin sensitivity (Khasawneh et al., 2009). Thus, the chronic, low-grade inflammation associated with obesity may contribute not only to the development of metabolic diseases such as diabetes, but also to cancer.

Conclusion

High levels of cellular nutrient metabolism result in ROS production and oxidative stress that can contribute to the development and progression of cancer. While this frequently occurs as a result of oncogene activation, it is becoming evident that organismal over-nutrition also leads to cellular stress responses that promote carcinogenesis. The obesity epidemic does not seem to be abating and contributes significantly to the cancer burden. At present, it appears likely that obesity can promote cancer development through a combination of hyperinsulinemia and nutrient-stress induced chronic low-grade inflammation, with each of these factors playing lesser or greater roles in different cancer types. It is likely that additional mechanisms also participate in linking chronic over-nutrition with cancer development and progression. For example, it will be interesting to examine how nutritionally-induced changes in cell surface proteins and their glycosylation regulate tumor cell response to immune cells and the microenvironment. Additionally, assessing which specific proteins are differentially modified by acetylation and/or O-

GlcNAcylation during conditions of nutrient excess might provide insight into the potential roles of these modifications in the development of cancer in obesity. Over the past several years, our knowledge of the interaction between metabolic and signaling pathways and the metabolic changes associated with cancer has increased dramatically, suggesting that the time is right to address how altered metabolism promotes cancer in the context of metabolic disease.

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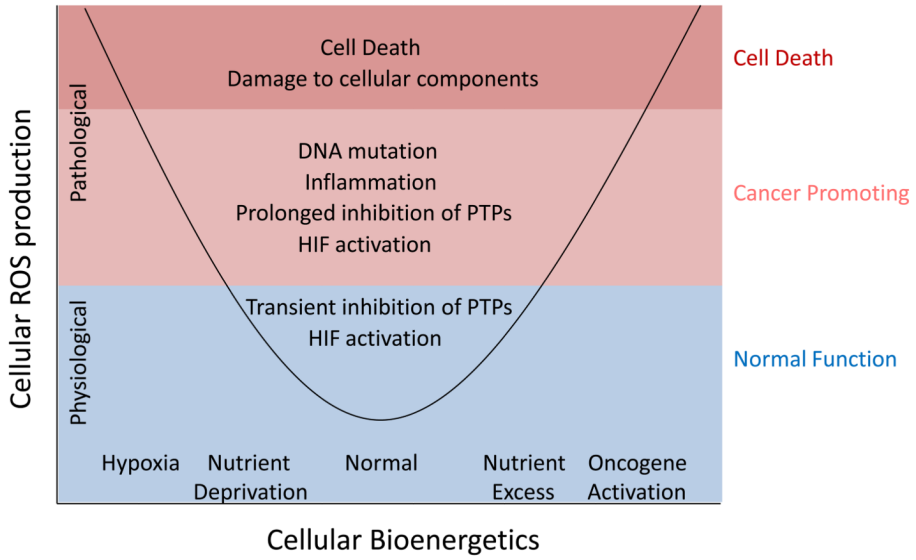

Figure 1. Both nutrient deficiency and nutrient excess can cause cellular stress

Mitochondrial ROS production from the electron transport chain increases in response to either hypoxia or oncogene activation and nutrient excess. Other mitochondrial sources of ROS such as proline oxidase are also involved in stress responses. At low levels, ROS production is critical for normal physiological processes, such as proliferation and differentiation, through regulation of signaling. At higher levels, ROS can induce changes that promote the development of cancer, such as mutation of DNA, prolonged signaling, and activation of inflammatory pathways. High levels of ROS can also lead to irreversible damage to cellular components and cell death.

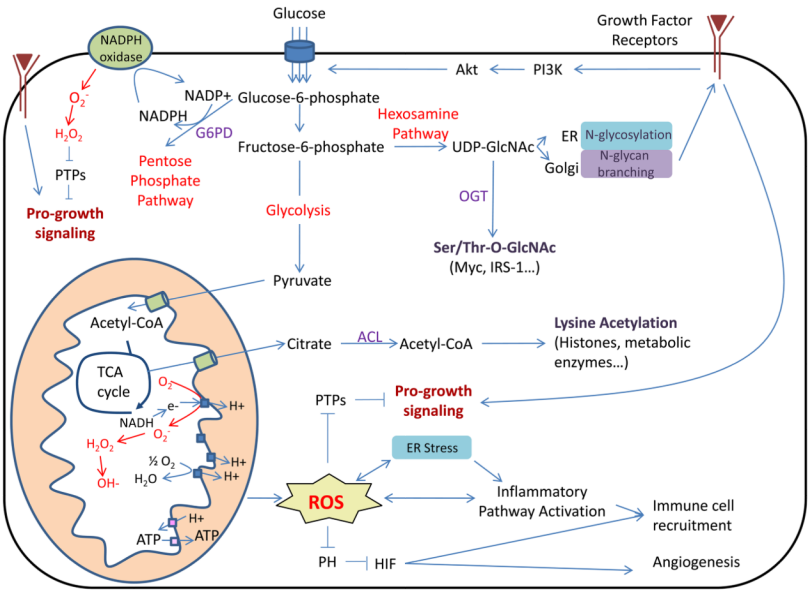

Figure 2. High levels of nutrient uptake induced by chronic growth factor signaling or oncogenic mutations cause cellular ROS stress and can contribute to cancer development through multiple mechanisms

Elevated mitochondrial or NADPH oxidase-dependent ROS production can contribute to cancer through multiple mechanisms, including DNA mutation, activation of inflammatory pathways, HIF activation, and promotion of pro-growth signaling. ER stress can also activate inflammatory pathways and regulate oxidative stress response. Increased glucose metabolism leading to changes in protein acetylation and O-GlcNAcylation can regulate signaling, transcription, and metabolism. Metabolic regulation of N-glycan branching modulates growth factor receptor surface expression and downstream signaling. Increased pro-growth signaling, HIF stabilization, or mutation resulting in oncogene activation can all promote increased glucose uptake and metabolism, leading to a vicious cycle of increased nutrient uptake, stress responses, and cancer promotion.

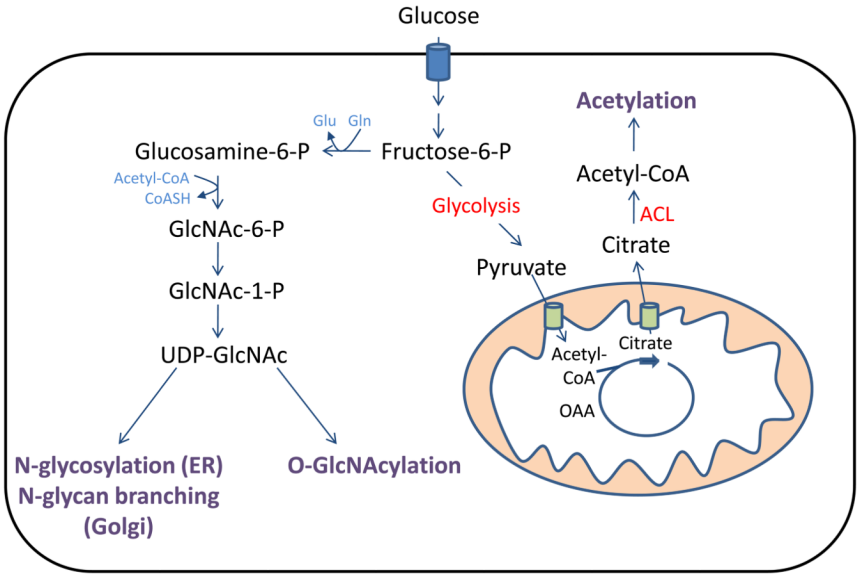

Figure 3. Metabolically-sensitive protein modifications can participate in cellular nutrient sensing

Production of acetyl-CoA for acetylation is regulated by ATP-citrate lyase (ACL), in a manner dependent on the availability of mitochondria-derived citrate. Both N-linked glycosylation and O-GlcNAcylation of proteins relies on production of UDP-GlcNAc by the hexosamine biosynthetic pathway.