Chapter 4

c-Myc Metabolic Addiction in Cancers Counteracted by Resveratrol and NQO2

Tze-chen Hsieh, Barbara B. Doonan and Joseph M. Wu

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77852

Abstract

Transcription factor c-myc is frequently amplified/overexpressed in human cancers. One event c-myc controls is metabolic reprogramming or the addiction for glucose and/or glutamine as nutrients. Rewiring of metabolic circuitry provides cancer cells with a gain-of-survival advantage. Accordingly, the aversion of two types of oncogenic-distinct metabolic addictions via c-myc control offers an anti-tumorigenic approach. Resveratrol reportedly inhibits the uptake/transport of glucose or glutamine and reduces c-myc expression in cancer cells. Whether c-myc control by resveratrol involves quinone reductase NQO2 is unknown. NQO2 expressing (shRNA08) and knockdown (shRNA25) CWR22Rv1 prostate cancer cells were generated and used to study the role of NQO2 in growth and cell cycle control. Immunoblot analyses were used to evaluate the changes of cell cycle-associated proteins. NQO2 in mediating degradation of cyclin D1 via AKT/GSK-3β by resveratrol was tested by determining AKT and chymotrypsin-like proteasome activities. Molecular modeling and pull-down/deletion assays were used to evaluate the interaction between NQO2 and AKT. Resveratrol interacts with NQO2, a quinone reductase that plays a key role in resveratrol-induced AKT/GSK3β-mediated degradation of cyclin D1. In this chapter, we unravel control of expression and stability of c-myc by the resveratrol-NQO2 axis as an approach to overcome c-myc-mediated metabolic reprogramming.

Keywords: resveratrol, c-myc, NQO2, metabolic addiction

1. Introduction

The c-myc oncoprotein is a well-studied, multifunctional transcription factor that controls cell proliferation, metabolism, and stress responses [1, 2]. Deregulation (amplification/overexpression) of c-myc occurs in many human cancers and is considered a "transition gatekeeper" for tumorigenesis. c-Myc controls metabolic reprogramming, a key event in tumorigenesis that provides

gain-of-survival adaptive advantage, enabling cancers to thrive in tumor microenvironments with limited nutrient resources [3–5]. This rewiring of metabolic circuitry is characterized by (i) preferred use of glucose to produce ATP by aerobic glycolysis combined with increased lactate production even in oxygen abundance and (ii) dependence on glutamine for conversion to glutamate and subsequent entry into the tricarboxylic acid cycle by anaplerosis [6, 7]. The metabolic calibration by c-myc in cancer cells is orchestrated to adequately meet the demands of energy expenditure, macromolecular syntheses, and disposal of catabolic wastes, in concordance with robust cell growth. For example, the capacity for aerobic glycolysis in highly proliferative cancer cells is controlled by c-myc via an increase in glucose uptake as a result of induced expression of glucose transporter 1 (Glut1) and lactate dehydrogenase (LDH) to stimulate glycolytic flux [8–12]. Similarly, to accommodate the utilization of glutamine, c-myc upregulates the level of expression of sodium-dependent neutral amino acid transporter (SLC1A5), thereby facilitating the transport of glutamate and uptake of glutamine. c-Myc also induces glutaminase GLS which catalyzes the conversion of glutamine to glutamate [8–10]. We hypothesize that the inhibition of c-myc expression and function, in addition to restriction of access to glucose/glutamine are both bona fide anti-carcinogenic approaches.

2. Metabolic addiction in malignant tumors presents new targets for cancer therapy

Cancer biology studies, for the past several decades, have focused on the identification and defining of DNA mutations, the narrowly centered focus being underpinned by the viewpoint that cancer is largely a genetic disease. However, cancer cells are also endowed by the acquisition of several hallmarks that enable them to become tumorigenic; among them is the apparent rewiring of gene:metabolite circuitry coordinating an altered metabolic adaptation with dysregulated cell proliferation in cancer cells [13]. Thus, the ability of cancer cells to preferentially utilize glucose and aerobic glycolysis as a paradoxical coincidence with ample oxygen supply was historically made by Warburg in the 1920s [14]. The so-called Warburg phenomenon amply illustrates the oncogenic addiction for glucose as a rapidly established metabolic adaptation that increases the capacity for ATP generation to meet the energy demands for unrestricted tumor cell proliferation and metastasis [7, 13, 15–17]. A similarly disproportionate reliance on amino acid glutamine is found in various cancer types [3, 4], compared to normal cells [18, 19]. The elevated levels of glutamine metabolism in cancer provide nitrogen for nucleotide and amino acid biosynthesis, and additionally, direct citrate and isocitrate in the TCA cycle for use in lipid synthesis, namely, reprogramming glutamine for energy production and for biosynthetic reactions via anaplerosis [6, 7, 20]. This metabolic rewiring is now recognized as an “Achilles heel” for cancer therapy.

3. c-Myc regulates metabolic addiction, cell growth and tumorigenesis

c-Myc is a transcription factor that controls the expression of a large number of genes including those involved in ribosome and mitochondria biogenesis, glucose and glutamine metabolism,

and lipid biosynthesis [8, 11, 12, 21]. c-Myc controlled gene sets underpin bioenergetics and synthesis of building blocks required for macromolecular assembly, transformation, proliferation and tumorigenesis. As a proto-oncogene, c-myc is frequently amplified/overexpressed in human cancers. Among a myriad of tumorigenic changes, c-myc controls are metabolic reprogramming—preferred use of glucose and glutamine, and sequential regulation of downstream targets/effectors [8, 11, 12, 21, 22]. c-Myc has been shown to induce the expression of glucose transporter Glut1 and glycolytic enzyme LDH to increase glycolytic flux. Similarly, c-myc also activates glutamine transporters SLC1A5 (also known as solute carrier family 1, member 5 and SLC38A5 - solute carrier family 38, member 5) that acts to increase uptake/transport of glutamine [12]. The rewiring of metabolic programming by c-myc provides cancer cells with survival advantage by meeting the demands of energy expenditure, macromolecular syntheses, and catabolic waste disposal. Studies have also shown that upregulation of c-myc is associated with an increase in mitochondrial glutaminolysis, which plays an overarching role on glutamine addiction in cancer cells. c-Myc also induces GLS expression through a dual negative mechanism – suppression of miR-23a/b by c-myc, and inhibition of GLS expression by miR-23a/b [21, 23]. The upregulation of GLS by c-myc facilitates the coordination of metabolic addiction with oncogenesis and the coalescence of metabolic calibration for survival with cellular transformation, proliferation, and cancer-related gene mutations. While metabolic addiction is clinically viewed as favoring carcinogenesis, it is equally plausible that the same nutrient dependence may become a restriction point for designing cancer therapy. Specifically, by limiting access to nutrients within the tumor microenvironments, novel oncogenic targets could become evident and are amenable for rational design of countermeasure strategies. A notable example can be found in the control of c-myc as a "master driver" for cancer; an equally appealing consideration likely involves the downstream targets regulated by c-myc [24, 25].

Resveratrol is a grape polyphenol whose efficacy has been amply supported by tissue culture and animal studies, and in limited clinical trials [26–30]. Resveratrol has been reported to inhibit c-myc in multiple cancer types including medulloblastoma cells, breast cancer cells and osteosarcoma [31–33]. How resveratrol inhibits c-myc via rewiring of metabolite:gene circuitry in tumorigenesis remains largely unknown and will be discussed in the next section. Greater understanding of this will add yet another dimension to the cancer preventive and therapeutic efficacy of resveratrol.

4. Aversion of c-myc metabolic addiction using resveratrol and its target protein NQO2 is an untested anti-tumorigenic approach

Since c-myc is involved in control of metabolic processing and resveratrol can inhibit c-myc expression, we propose that the c-myc-mediated metabolic dysregulation of cancer can be countered using grape-derived resveratrol. It is our hypothesis that resveratrol exerts a dual role in disrupting c-myc-mediated cancer metabolic reprogramming—a **direct effect** impinging on c-myc expression and stability and associated uptake/transport of glucose and/or glutamine, and an **indirect effect** involving NQO2 as the mediator affecting c-myc stability via AKT, its downstream effector GSK3β, and by extension control of the activity and function of the proteasome. This provocative assignment for NQO2 greatly expands its cellular role from a cytosolic flavoprotein discovered in 1961 and classically considered phase II enzyme to a multi-tasking regulator involved in cancer cell metabolic reprogramming. Ample data support this postulation.

NQO2 requires NRH (N-ribosyl dihydronicotinamide) as the cosubstrate for catalysis; the biosynthetic source of NRH in mammalian cells has not been elucidated suggesting that NQO2 may have other novel cellular functions. Mouse keratinocyte studies show that NQO2 controls TNF-induced NF-kB activation; NQO2 deletion potentiates the induction of apoptosis by abolishing TNF-induced cell survival kinases including JNK, AKT, p38, and p44/p42 MAPK [34]. NQO2 stabilizes C/EBPα degradation mediated by 20S proteasomes [35]. We previously showed that NQO2 is a high affinity target protein of resveratrol: NQO2 binds resveratrol with $K_D \leq 50$ nM [36]; X-ray crystal analysis shows that binding to resveratrol occurs in a hydrophobic pocket located between dimeric NQO2, possibly where the cosubstrate NRH binds [37]. Since the plasma concentration of resveratrol in humans can reach from 0.5 μM [38] to as high as 4 μM [39, 40], it may be suggested that *in vivo* levels of resveratrol are sufficient for binding and inhibiting NQO2 enzyme activity and modulation of its other functions. One such novel role may pertain to control of c-myc turnover *via* T58 phosphorylation by AKT/GSK3β [41–43], results which agree with/support/resemble our findings using NQO2-knockdown CWR22Rv1 cells, showing that NQO2: (i) inhibits AKT activity and (ii) controls cyclin D1 stability via AKT/GSK3β mediated threonine T-286 phosphorylation [44]. Moreover, oxidized and reduced NQO2 was recently reported to selectively bind DNA-intercalating agents, including ethidium bromide, acridine orange, and doxorubicin; all three agents functioning as inhibitors at nanomolar levels, thereby raising the provocative tenet that NQO2 is a potential regulator of eukaryotic gene transcription and expression [45]. Accordingly, activators/inhibitors of NQO2 may be developed as drug targets for the management of cancers harboring amplified/overexpressed transcriptional factor c-myc. As hypothesized, inhibitors of NQO2 could modify the interplay between NQO2 and c-myc and disrupt the c-myc-mediated growth advantage in cancer cells. If c-myc control is shown to be connected to and under the rubric of genetic (NQO2) and/or chemical (resveratrol) mediated control in glucose/glutamine addiction cancer, then the control of NQO2-c-myc axis by resveratrol may be a promising cancer preventative and therapeutic lead, providing insights on how to better manage and treat glucose/glutamine addicted diseases.

5. Control of c-myc stability by NQO2

As a powerful factor governing the transcription of large gene sets that encode proteins playing critical roles in numerous cellular processes, both in normal and diseased states, the level of expression of c-myc is under stringent control. Ample data point to c-myc degradation being regulated by sequential phosphorylation of S62 and T58, by two external signal activated kinase cascades, respectively, the RAF/MEK/ERK and PI3K/AKT/GSK3β signaling pathways [46–51]. T58 phosphorylation of c-myc promotes its interaction with the ubiquitin ligases Fbw7 and Skp2, ubiquitination and degradation by the proteasome [52–54]. Additionally, deubiquitinating enzymes, USP28 and USP36, also contribute to c-myc degradation [54–56]. Of note, the reported AKT/GSK3β-mediated c-myc T58 phosphorylation in control of its turnover [41–43] is relevant to our own studies: (i) NQO2 is involved in AKT/GSK3β-mediated cyclin D1 T286 phosphorylation and degradation and (ii) NQO2 knockdown CWR22Rv1-sh25 cells show a 37% decrease in chymotrypsin-like proteasome activity

compared to control CWR22Rv1-sh08 cells [44]. These results suggest a hitherto-never-considered aspect of control of c-myc stability by NQO2. Next, we will discuss a proposed study to test the potential role NQO2 plays as the mediator of control of c-myc stability via AKT/GSK3β-c-myc T-58 phosphorylation, and by regulation of activity and functioning of the proteasome.

Our previous studies showing that resveratrol exerts its effects via its target protein NQO2 provide the impetus for testing that down regulation of c-myc by resveratrol requires the participation of NQO2. Based on our data that NQO2 affects cyclin D1 turnover, we expect that NQO2 will increase c-myc degradation, that is, $\downarrow t_{1/2}$ instead of conferring protection on c-myc stability by competing against proteasome as has been reported for C/EBPα whose degradation by 20S proteasome is attenuated by NQO2 [35]. Since c-myc degradation by proteasomes is known to involve a multitude of mechanisms, there is a possibility resveratrol or NQO2 may directly affect c-myc degradation by exerting control on ubiquitin ligases like Fbw7 and Skp2 or the deubiquitinating enzyme, USP28. As to whether NQO2 interacts with AKT to affect AKT-GSK3β-mediated c-myc degradation, our expectation is that the accumulated c-myc protein in MG132-treated cells will show a higher T58 phosphorylation as compared to nontreated control cells. As a corollary, addition of GSK3β inhibitor LiCl to treated cells should significantly reduce T58 phosphorylated c-myc protein, in parallel with an increase in c-myc protein accumulation. siRNA-knockdown of GSK-3α or -3β in NQO2 expression cells compared to NQO2 knockdown cells will further confirm the role of GSK3β in mediating c-myc degradation. Results of these studies will provide support for the as yet untested hypothesis regarding the indirect role of NQO2 in controlling AKT → GSK3β → c-myc T58 phosphorylation → c-myc degradation by proteasome, and the direct role of resveratrol acting as a metabolic switch to shut off c-myc-mediated metabolic reprogramming in cancer cells.

6. Control of c-myc stability by resveratrol may involve the stimulation of proteasome β5 subunit

Epidemiological studies have shown that moderate intake of red wine is correlated with a reduced incidence of dementia and neurodegenerative disease [57]. Moreover, resveratrol, a tri-hydroxyl stilbene found in abundance in red wine, red grapes, peanuts and a number of other consumed foods in the United States, has been reported to confer protection against oxidative stress in PC-12 cells [58, 59]. It has been determined that the preeminent presence of senile plaques, composed mainly of amyloid-β (Aβ) deposits, is a pathological brain feature in individuals diagnosed with Alzheimer's Disease (AD). The Aβ peptides are derived from cleavage of the amyloid-β precursor protein (APP) and have been shown to destabilize neurons and lead to cell death through the induction of oxidative stress, mediated by the generation of reactive oxygen species (ROS) and elevation in intracellular hydrogen peroxide [60–62]. Compelling evidence supports that Aβ peptides serve as the "primary instigator" of AD [63]. Davies and coworkers[64] used tissue culture studies

combined with biochemical assays and siRNA-silencing approaches to show that resveratrol lowered the levels of secreted and intracellular Aβ-peptides in a concentration and time-dependent manner. Further, this effect occurred not by targeting the Aβ-producing enzymes β and γ-secretases or by affecting the stability of APP or the turnover of its C-terminal fragments; but instead appeared to involve the promotion of intracellular degradation of Aβ via a proteasome subunit β5-dependent mechanism. It is worth noting that resveratrol reportedly also promotes the proteasome-mediated degradation of important regulatory proteins, including cyclin D1 [65], the estrogen receptor-α [66], and the hypoxia-inducible factor-1α [67].

7. c-Myc as an IDP (intrinsically disordered protein) may facilitate its turnover by resveratrol via a ubiquitin-independent, 20S proteasome-mediated mechanism

The notion that a folded three-dimensional structure is required for the biological function of a protein is dispelled by the discovery of intrinsically disordered proteins (IDPs) in the 1990s [68]. IDPs can be viewed as proteins that have minimal structures or are devoid of an overall defined fold, either entirely or in parts and are more likely to exist in dynamic, mosaic states under physiological conditions. The absence of structural orderliness also confers plasticity and "fussiness" to IDPs for diverse protein-protein interactions; however, the very same structural flexibility may also render them difficult and challenging as druggable targets using traditional structure-function drug design approaches.

Eukaryotic transcription factors perform important biological functions in control of gene expression. They play an essential role in identifying the target sequences on DNA located in the vicinity as well as far removed from the transcription start site through direct protein:nucleic acid interaction, and also are required for binding to a large array of co-transcription regulatory proteins via protein:protein interaction. As such, eukaryotic transcription factors have been shown to exhibit a high degree of intrinsic disorderliness; based on bioinformatic model prediction analysis it is estimated that more than 49% of human transcription factors contain intrinsic disorderliness [69]. Studies have also revealed that IDPs, because of their intrinsic destabilized nature circumventing the requirement for unfolding protein substrates for proteolysis by the 26S proteasome, are more likely to be degraded via an ubiquitin-independent mechanism using the 20S proteasomes [70].

The intrinsically unstructured protein (IUP), disorderly theme is also found in transcription factor c-myc; indeed, c-myc only attains an ordered structure after binding to its disordered partner MAX protein (myc-associated factor). Bioinformatics and experimental approaches have estimated that c-myc contains more than 45% of residues which have high probability for disordered structure formation [71]. The possession of intrinsically disordered regions allows c-myc to be degraded independently of ubiquitin, which may account for its observed short half-life at the mRNA [72–74] and protein levels [75–77], and is dynamically

aligned with its switch on/off master transcriptional role independent of collaborative interaction with the pool of cellular ubiquitin that drives 26S proteasome-mediated protein degradation.

It should be noted that the function of the 20S proteasome can also be modulated by interaction with NAD(P)H:quinone oxidoreductase 1 and 2 (NQO1 and NQO2). Previous studies have reported that NQO1 physically binds the 20S proteasome in an NADH-dependent manner [78–80] and to protect IDPs from degradation [81]. A double negative feedback mechanism exists between NQO1 and the 20S proteasome [78]. On the one hand, NQO1 acts to attenuate the proteolytic activity of the proteasome; on the other hand, the proteasome degrades the NQO1 FAD-free apo form which manifests as a partially unfolded structure and a substrate for the 20S proteasome [81]. Studies have shown that NQO2 confers protection against proteolytic degradation by the 20S proteasome [35, 82] albeit by a mechanism independent of NQO1 [83].

8. Conclusion

Resveratrol has been shown to inhibit the uptake/transport of glucose or glutamine, and decrease the expression of c-myc in cancer cells. Rewiring of metabolite:gene circuitry is a key event in tumorigenesis that has been known for decades [7, 15–17], however, with an incompletely understood underlying mechanism. In this chapter, we discuss the aversion of c-myc-mediated reprogrammed cancer cell metabolism by targeting the expression and stability of c-myc using a chemical/genetic disruptive approach focusing on resveratrol and its high affinity target NQO2 we identified [36]. Additionally, the hypothesis we propose broadens the classical function of NQO2 in quinone detoxification to AKT/c-myc-mediated metabolic reprogramming observable in a clinical setting. Taken together, resveratrol/NQO2 in a c-myc controlling role to block metabolic addiction represents a novel diet-based chemoprevention approach in concept, and is transformative in implications warranting further investigation. The results will lay foundation for discovery of drugs able to disrupt AKT/c-myc-mediated reorganized metabolism using NQO2 inhibitors.

In summary, we advance the thesis to avert c-myc-mediated metabolic reprogramming in cancer cells by targeting the control of c-myc and the uptake and metabolism of glucose and glutamine and their downstream effectors using resveratrol. We propose to focus on control of phosphorylation of c-myc T58 by GSK3β, shown to be critical for proteasome mediated c-myc degradation, by the resveratrol target protein NQO2 which we have previously shown to act as a modulator of AKT/GSK3β proteasome mediated degradation of cyclin D1 [44].

The dual role resveratrol plays in disrupting c-myc-mediated metabolic reprogramming in cancer cells—a direct role targeting suppression of c-myc expression and an indirect role involving NQO2-AKT-GSK 3β mediated increase in c-myc T58-phosphorylation for increased degradation by proteasome—is illustrated in **Figure 1**.

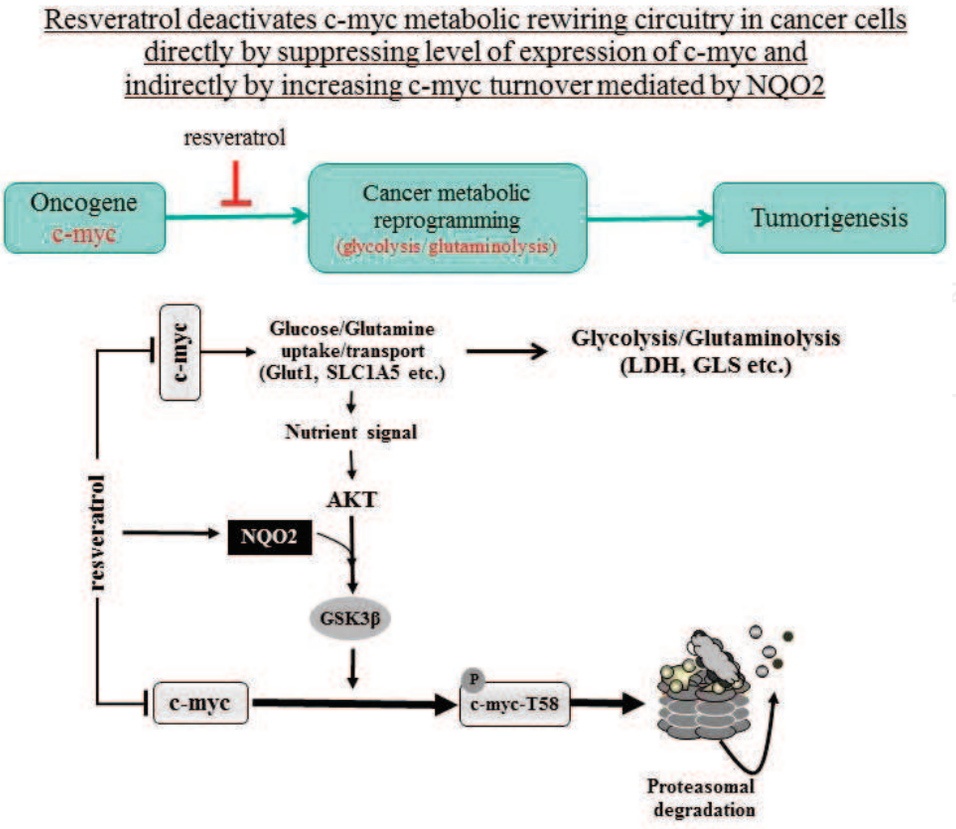

Figure 1. Resveratrol and NQO2 exert dual control of c-myc-mediated glucose/glutamine adaptation in cancer cells by transcription/translation suppression of c-myc expression and by control of proteasome-dependent c-myc stability according to the sequence: NQO2 binds AKT, reducing AKT kinase and increasing GSK 3β activity, resulting in increase in c-myc T58 phosphorylation and facilitating an increase in c-myc degradation by proteasome.

Author details

Tze-chen Hsieh, Barbara B. Doonan and Joseph M. Wu*

*Address all correspondence to: joseph_wu@nymc.edu

Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, New York, USA

References

[1] Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: Glutamine metabolism to cancer therapy. Nature Reviews. Cancer. 2016;**16**:619-634

[2] Stine ZE, Walton ZE, Altman BJ, Hsieh AL, Dang CV. MYC, metabolism, and cancer. Cancer Discovery. 2015;**5**:1024-1039

[3] Ward PS, Thompson CB. Metabolic reprogramming: A cancer hallmark even Warburg did not anticipate. Cancer Cell. 2012;**21**:297-308

[4] Hsieh AL, Walton ZE, Altman BJ, Stine ZE, Dang CV. MYC and metabolism on the path to cancer. Seminars in Cell & Developmental Biology. 2015;**43**:11-21

[5] Tarrado-Castellarnau M, de Atauri P, Cascante M. Oncogenic regulation of tumor metabolic reprogramming. Oncotarget. 2016;**7**:62726-62753

[6] Yang C, Harrison C, Jin ES, Chuang DT, Sherry AD, Malloy CR, et al. Simultaneous steady-state and dynamic 13C NMR can differentiate alternative routes of pyruvate metabolism in living cancer cells. The Journal of Biological Chemistry. 2014;**289**:6212-6224

[7] Smith B, Schafer XL, Ambeskovic A, Spencer CM, Land H, Munger J. Addiction to coupling of the Warburg effect with glutamine catabolism in Cancer cells. Cell Reports. 2016;**17**:821-836

[8] Dang CV. MYC, metabolism, cell growth, and tumorigenesis. Cold Spring Harbor Perspectives in Medicine. 2013;**3**

[9] Boroughs LK, DeBerardinis RJ. Metabolic pathways promoting cancer cell survival and growth. Nature Cell Biology. 2015;**17**:351-359

[10] Ferrer CM, Sodi VL, Reginato MJ. O-GlcNAcylation in cancer biology: Linking metabolism and signaling. Journal of Molecular Biology. 2016;**428**:3282-3294

[11] Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. Immunity. 2011;**35**:871-882

[12] Li B, Simon MC. Molecular pathways: Targeting MYC-induced metabolic reprogramming and oncogenic stress in cancer. Clinical Cancer Research. 2013;**19**:5835-5841

[13] Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011;**144**: 646-674

[14] Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. The Journal of General Physiology. 1927;**8**:519-530

[15] Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: The metabolic requirements of cell proliferation. Science. 2009;**324**:1029-1033

[16] Green DR, Galluzzi L, Kroemer G. Cell biology. Metabolic control of cell death. Science. 2014;**345**:1250256

[17] Potter M, Newport E, Morten KJ. The Warburg effect: 80 years on. Biochemical Society Transactions. 2016;**44**:1499-1505

[18] Wise DR, Thompson CB. Glutamine addiction: A new therapeutic target in cancer. Trends in Biochemical Sciences. 2010;**35**:427-433

[19] Lu W, Pelicano H, Huang P. Cancer metabolism: Is glutamine sweeter than glucose? Cancer Cell. 2010;**18**:199-200

[20] DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S, et al. Beyond aerobic glycolysis: Transformed cells can engage in glutamine metabolism that exceeds

the requirement for protein and nucleotide synthesis. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**:19345-19350

[21] Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, et al. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**:18782-18787

[22] Zwaans BM, Lombard DB. Interplay between sirtuins, MYC and hypoxia-inducible factor in cancer-associated metabolic reprogramming. Disease Models & Mechanisms. 2014;**7**:1023-1032

[23] Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, et al. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature. 2009;**458**:762-765

[24] Wang JB, Erickson JW, Fuji R, Ramachandran S, Gao P, Dinavahi R, et al. Targeting mitochondrial glutaminase activity inhibits oncogenic transformation. Cancer Cell. 2010;**18**: 207-219

[25] Hensley CT, Wasti AT, DeBerardinis RJ. Glutamine and cancer: Cell biology, physiology, and clinical opportunities. The Journal of Clinical Investigation. 2013;**123**:3678-3684

[26] Park EJ, Pezzuto JM. The pharmacology of resveratrol in animals and humans. Biochimica et Biophysica Acta. 1852;**2015**:1071-1113

[27] Carter LG, D'Orazio JA, Pearson KJ. Resveratrol and cancer: Focus on in vivo evidence. Endocrine-Related Cancer. 2014;**21**:R209-R225

[28] Yang X, Li X, Ren J. From French paradox to cancer treatment: Anti-cancer activities and mechanisms of resveratrol. Anti-Cancer Agents in Medicinal Chemistry. 2014;**14**:806-825

[29] Scott E, Steward WP, Gescher AJ, Brown K. Resveratrol in human cancer chemoprevention – Choosing the 'right' dose. Molecular Nutrition & Food Research. 2012;**56**:7-13

[30] Smoliga JM, Baur JA, Hausenblas HA. Resveratrol and health – A comprehensive review of human clinical trials. Molecular Nutrition & Food Research. 2011;**55**:1129-1141

[31] Zhang P, Li H, Wu ML, Chen XY, Kong QY, Wang XW, et al. c-Myc downregulation: A critical molecular event in resveratrol-induced cell cycle arrest and apoptosis of human medulloblastoma cells. Journal of Neuro-Oncology. 2006;**80**:123-131

[32] Zhou J, Zhou M, Yang FF, Liu CY, Pan RL, Chang Q, et al. Involvement of the inhibition of intestinal glucuronidation in enhancing the oral bioavailability of resveratrol by labrasol containing nanoemulsions. Molecular Pharmaceutics. 2015;**12**:1084-1095

[33] Xie D, Zheng GZ, Xie P, Zhang QH, Lin FX, Chang B, et al. Antitumor activity of resveratrol against human osteosarcoma cells: A key role of Cx43 and Wnt/β-catenin signaling pathway. Oncotarget. 2017;**8**:111419-111432

[34] Ahn KS, Gong X, Sethi G, Chaturvedi MM, Jaiswal AK, Aggarwal BB. Deficiency of NRH:quinone oxidoreductase 2 differentially regulates TNF signaling in keratinocytes:

Up-regulation of apoptosis correlates with down-regulation of cell survival kinases. Cancer Research. 2007;**67**:10004-10011

[35] Xu J, Patrick BA, Jaiswal AK. NRH:quinone oxidoreductase 2 (NQO2) protein competes with the 20 S proteasome to stabilize transcription factor CCAAT enhancer-binding protein α (C/EBPα), leading to protection against γ radiation-induced myeloproliferative disease. The Journal of Biological Chemistry. 2013;**288**:34799-34808

[36] Buryanovskyy L, Fu Y, Boyd M, Ma Y, Hsieh TC, Wu JM, et al. Crystal structure of quinone reductase 2 in complex with resveratrol. Biochemistry. 2004;**43**:11417-11426

[37] Yan C, Dufour M, Siegel D, Reigan P, Gomez J, Shieh B, et al. Indolequinone inhibitors of NRH:quinone oxidoreductase 2. Characterization of the mechanism of inhibition in both cell-free and cellular systems. Biochemistry. 2011;**50**:6678-6688

[38] Boocock DJ, Faust GE, Patel KR, Schinas AM, Brown VA, Ducharme MP, et al. Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. Cancer Epidemiology, Biomarkers & Prevention. 2007;**16**:1246-1252

[39] Vitrac X, Bornet A, Vanderlinde R, Valls J, Richard T, Delaunay JC, et al. Determination of stilbenes (delta-viniferin, trans-astringin, trans-piceid, cis-and trans-resveratrol, epsilon-viniferin) in Brazilian wines. Journal of Agricultural and Food Chemistry. 2005;**53**: 5664-5669

[40] Opie LH, Lecour S. The red wine hypothesis: From concepts to protective signalling molecules. European Heart Journal. 2007;**28**:1683-1693

[41] Yeh E, Cunningham M, Arnold H, Chasse D, Monteith T, Ivaldi G, et al. A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells. Nature Cell Biology. 2004;**6**:308-318

[42] Schülein C, Eilers M. An unsteady scaffold for Myc. The EMBO Journal. 2009;**28**:453-454

[43] Bechard M, Dalton S. Subcellular localization of glycogen synthase kinase 3beta controls embryonic stem cell self-renewal. Molecular and Cellular Biology. 2009;**29**:2092-2104

[44] Hsieh TC, Yang CJ, Lin CY, Lee YS, Wu JM. Control of stability of cyclin D1 by quinone reductase 2 in CWR22Rv1 prostate cancer cells. Carcinogenesis. 2012;**33**:670-677

[45] Leung KK, Shilton BH. Binding of DNA-intercalating agents to oxidized and reduced quinone reductase 2. Biochemistry. 2015;**54**:7438-7448

[46] Sears R, Nuckolls F, Haura E, Taya Y, Tamai K, Nevins JR. Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. Genes & Development. 2000;**14**: 2501-2514

[47] Adhikary S, Eilers M. Transcriptional regulation and transformation by Myc proteins. Nature Reviews. Molecular Cell Biology. 2005;**6**:635-645

[48] Eilers M, Eisenman RN. Myc's broad reach. Genes & Development. 2008;**22**:2755-2766

[49] Welcker M, Clurman BE. FBW7 ubiquitin ligase: A tumour suppressor at the crossroads of cell division, growth and differentiation. Nature Reviews. Cancer. 2008;**8**:83-93

[50] Silva A, Wang J, Lomahan S, Tran TA, Grenlin L, Suganami A, et al. Aurora kinase a is a possible target of OSU-03012 to destabilize MYC family proteins. Oncology Reports. 2014;**32**:901-905

[51] Davis RJ, Welcker M, Clurman BE. Tumor suppression by the Fbw7 ubiquitin ligase: Mechanisms and opportunities. Cancer Cell. 2014;**26**:455-464

[52] Yada M, Hatakeyama S, Kamura T, Nishiyama M, Tsunematsu R, Imaki H, et al. Phosphorylation-dependent degradation of c-Myc is mediated by the F-box protein Fbw7. The EMBO Journal. 2004;**23**:2116-2125

[53] Farrell AS, Sears RC. MYC degradation. Cold Spring Harbor Perspectives in Medicine. 2014;**4**

[54] Sun XX, Sears RC, Dai MS. Deubiquitinating c-Myc: USP36 steps up in the nucleolus. Cell Cycle. 2015;**14**:3786-3793

[55] Popov N, Wanzel M, Madiredjo M, Zhang D, Beijersbergen R, Bernards R, et al. The ubiquitin-specific protease USP28 is required for MYC stability. Nature Cell Biology. 2007;**9**:765-774

[56] Amati B, Sanchez-Arévalo Lobo VJ. MYC degradation: deubiquitinating enzymes enter the dance. Nature Cell Biology. 2007;**9**:729-731

[57] Russo A, Palumbo M, Aliano C, Lempereur L, Scoto G, Renis M. Red wine micronutrients as protective agents in Alzheimer-like induced insult. Life Sciences. 2003;**72**:2369-2379

[58] Jang JH, Surh YJ. Protective effect of resveratrol on beta-amyloid-induced oxidative PC12 cell death. Free Radical Biology & Medicine. 2003;**34**:1100-1110

[59] Virgili M, Contestabile A. Partial neuroprotection of in vivo excitotoxic brain damage by chronic administration of the red wine antioxidant agent, trans-resveratrol in rats. Neuroscience Letters. 2000;**281**:123-126

[60] Morais Cardoso S, Swerdlow RH, Oliveira CR. Induction of cytochrome c-mediated apoptosis by amyloid beta 25-35 requires functional mitochondria. Brain Research. 2002;**931**: 117-125

[61] Behl C, Davis JB, Lesley R, Schubert D. Hydrogen peroxide mediates amyloid beta protein toxicity. Cell. 1994;**77**:817-827

[62] Geula C, Wu CK, Saroff D, Lorenzo A, Yuan M, Yankner BA. Aging renders the brain vulnerable to amyloid beta-protein neurotoxicity. Nature Medicine. 1998;**4**:827-831

[63] Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. Science. 2002;**297**:353-356

[64] Marambaud P, Zhao H, Davies P. Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides. The Journal of Biological Chemistry. 2005;**280**:37377-37382

[65] Joe AK, Liu H, Suzui M, Vural ME, Xiao D, Weinstein IB. Resveratrol induces growth inhibition, S-phase arrest, apoptosis, and changes in biomarker expression in several human cancer cell lines. Clinical Cancer Research. 2002;**8**:893-903

[66] Pozo-Guisado E, Lorenzo-Benayas MJ, Fernández-Salguero PM. Resveratrol modulates the phosphoinositide 3-kinase pathway through an estrogen receptor alpha-dependent mechanism: Relevance in cell proliferation. International Journal of Cancer. 2004;**109**:167-173

[67] Cao Z, Fang J, Xia C, Shi X, Jiang BH. Trans-3,4,5′-Trihydroxystibene inhibits hypoxia-inducible factor 1alpha and vascular endothelial growth factor expression in human ovarian cancer cells. Clinical Cancer Research. 2004;**10**:5253-5263

[68] Gsponer J, Babu MM. The rules of disorder or why disorder rules. Progress in Biophysics and Molecular Biology. 2009;**99**:94-103

[69] Dunker AK, Uversky VN. Drugs for 'protein clouds': Targeting intrinsically disordered transcription factors. Current Opinion in Pharmacology. 2010;**10**:782-788

[70] Erales J, Coffino P. Ubiquitin-independent proteasomal degradation. Biochimica et Biophysica Acta. 2014;**1843**:216-221

[71] Dunker AK, Silman I, Uversky VN, Sussman JL. Function and structure of inherently disordered proteins. Current Opinion in Structural Biology. 2008;**18**:756-764

[72] Buckler AJ, Vie H, Sonenshein GE, Miller RA. Defective T lymphocytes in old mice. Diminished production of mature c-myc RNA after mitogen exposure not attributable to alterations in transcription or RNA stability. Journal of Immunology. 1988;**140**:2442-2446

[73] Lindsten T, June CH, Thompson CB. Multiple mechanisms regulate c-myc gene expression during normal T cell activation. The EMBO Journal. 1988;**7**:2787-2794

[74] Gamble DA, Schwab R, Weksler ME, Szabo P. Decreased steady state c-myc mRNA in activated T cell cultures from old humans is caused by a smaller proportion of T cells that transcribe the c-myc gene. Journal of Immunology. 1990;**144**:3569-3573

[75] Li Q, Kluz T, Sun H, Costa M. Mechanisms of c-myc degradation by nickel compounds and hypoxia. PLoS One. 2009;**4**:e8531

[76] Gustafson WC, Meyerowitz JG, Nekritz EA, Chen J, Benes C, Charron E, et al. Drugging MYCN through an allosteric transition in Aurora kinase A. Cancer Cell. 2014;**26**:414-427

[77] Li S, Jiang C, Pan J, Wang X, Jin J, Zhao L, et al. Regulation of c-Myc protein stability by proteasome activator REGγ. Cell Death and Differentiation. 2015;**22**:1000-1011

[78] Asher G, Tsvetkov P, Kahana C, Shaul Y. A mechanism of ubiquitin-independent proteasomal degradation of the tumor suppressors p53 and p73. Genes & Development. 2005;**19**:316-321

[79] Xu J, Jaiswal AK. NAD(P)H:quinone oxidoreductase 1 (NQO1) competes with 20S proteasome for binding with C/EBPα leading to its stabilization and protection against

radiation-induced myeloproliferative disease. The Journal of Biological Chemistry. 2012;**287**:41608-41618

[80] Adamovich Y, Shlomai A, Tsvetkov P, Umansky KB, Reuven N, Estall JL, et al. The protein level of PGC-1α, a key metabolic regulator, is controlled by NADH-NQO1. Molecular and Cellular Biology. 2013;**33**:2603-2613

[81] Moscovitz O, Tsvetkov P, Hazan N, Michaelevski I, Keisar H, Ben-Nissan G, et al. A mutually inhibitory feedback loop between the 20S proteasome and its regulator, NQO1. Molecular Cell. 2012;**47**:76-86

[82] Gong X, Kole L, Iskander K, Jaiswal AK. NRH:quinone oxidoreductase 2 and NAD(P)H:quinone oxidoreductase 1 protect tumor suppressor p53 against 20s proteasomal degradation leading to stabilization and activation of p53. Cancer Research. 2007;**67**:5380-5388

[83] Ben-Nissan G, Sharon M. Regulating the 20S proteasome ubiquitin-independent degradation pathway. Biomolecules. 2014;**4, 884**:862