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Metabolic regulation of sirtuins upon fasting and the implication for cancer

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Abstract

Purpose of review—The purpose of this review is to highlight recent studies on mammalian sirtuins that coordinately regulate cellular metabolic homeostasis upon fasting and to summarize the beneficial effects of fasting on carcinogenesis and cancer therapy.

Recent findings—Recent studies have demonstrated that fasting may protect normal cells and mice from the metabolic conditions that are harmful as well as decrease the incidence of carcinogenesis. Fasting could also slow the tumor growth and augment the efficacy of certain systemic agents/chemotherapy drugs in various cancers. The mechanism behind this proposed idea may be due to, at least in some part, the metabolic regulation by sirtuin family proteins whose functions are involved in specific aspects of longevity, stress response and metabolism. Sirtuins, particularly SIRT1 and SIRT3, can be activated by fasting and further exhibit their effects in insulin response, antioxidant defense, and glycolysis. Therefore, sirtuins may have anticancer effects by shifting metabolism to a less proliferative cell phenotype as well as less prone to oxidative stress attack.

Summary—The in-depth understanding of the essential role of sirtuins in the fasting process may have significant implications in developing a new metabolic diagram of cancer prevention or treatment.

Keywords

acetylation; acetylome; metabolism; SIRT3; sirtuins

INTRODCUTION

A fundamental observation in oncology is that the rate of malignancies increases significantly as a function of age, suggesting a potential mechanistic link between the cellular processes governing longevity and the development of cancers [1,2]. As such, of particular interest is how does the lifespan of an organism affect the molecular mechanisms

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Conflicts of interest

There are no conflicts of interest.

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of cancer development and can we use our deepening knowledge of aging to enhance prevention or treatment of cancer? It is very well established that there is a fundamental connection between diet and/or caloric intake and aging [3–5]. Thus, it seems reasonable to propose that there may be a mechanistic connection between diet and/or caloric intake, aging-related cell signaling processes, and the aberrant processes that promote carcinogenesis.

In this regard, recent studies have shown that fasting (restricted diet, intermittent fasting, and fasting) increases the lifespan both in unicellular yeast and mammals via downregulating conserved nutrient-sensitive signaling pathways/proteins as well as activating stress-resistant pathways [6]. In addition, this dietary intervention was also suggested to protect animals from many age-related late-onset diseases such as insulin resistance, neuro-degeneration and cancer as well as enhancing the chemotherapy effectiveness in cancer treatment [7,8▪▪]. More important, many pathways affected by fasting are related to cellular growth, metabolism and protection against oxidative stress, which are critical in cancer biology [6,9]. Given the importance of fasting or, perhaps better stated, nutrient stress, an important question is whether there is a master metabolic regulator involved in the fasting mediated biological effects. If this is the case, then it seems reasonable to propose that the manipulation of these nutrient-responsive signaling pathways related to aging and carcinogenesis through different downstream effectors may be molecular targets for new therapeutic interventions [10,11].

Sirtuins, a family of highly conserved NAD^+-dependent lysine-specific deacetylases has recently been suggested to have a critical impact in the direction and regulation of many metabolic pathways as well as energy sensing in mammals [12–14]. Furthermore, numerous studies have also demonstrated that sirtuins are involved in several tumor biological processes ranging from cellular energy homeostasis to reactive oxygen species (ROS) production [12–14,15▪▪]. Several research groups have shown that sirtuins are activated by responding to fasting, thus linking the nutrient signals with cellular metabolic pathways involved in cancer [16,17]. In this regard, sirtuins can act as cellular energy sensors and direct the cell to match energy needs to energy production and consumption and as such, it is proposed that these processes also may protect cells from a wide range of biological disorders including cancer by deacetylating target proteins in many key physiological and metabolic pathways[18,19]. Thus, this review mainly focuses on the recent advances on the role of sirtuins in cancer biology and describes the relation among fasting, the functions of sirtuins, and the implication for cancer therapy.

Fasting vs. calorie restriction

Nutrient deprivation or nutrient stress likely encompasses many forms of dietary restriction including calorie/dietary restriction, time restricted diet (tRD), intermittent fasting [20–22], and fasting. Calorie/ dietary restriction describes the reduction in calorie intake by usually 20–30%. A time-restricted diet describes one meal per day with normal daily calorie intake. Intemittent fasting is alternating days of regular diet and fasting, whereas pure fasting is complete food starvation for several consecutive days. Though calorie/dietary restriction has been shown to positively affect lifespan and cancer treatment in laboratory settings [23,24],

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translation to clinical studies has been limited for several reasons. First, chronic calorie/dietary restriction has been shown to only delay the progression of tumor growth and this delay will occur only for a subset of malignancies [25–27]. In addition, the weight loss and weakening of the immune system created by calorie/dietary restriction makes it difficult for cancer patients undergoing chemotherapy [28,29]. By contrast, a tRD and/or intermittent fasting exhibited similar effects compared to calorie/dietary restriction, but do not result in weight loss and can be more tolerable in patients [8▪▪,30]. The only disadvantage for tRD, intermittent fasting and even calorie/dietary restriction is that they require an extended time before any protection takes effect that may limit the potential use in cancer therapy.

Finally, fasting immediately prior to chemotherapy treatment followed by a return to the regular diet does not cause weight loss in the long term [7] while potentially enhancing the beneficial effects of chemotherapy [31]. However, is it really realistic for a cancer patient undergoing prolonged fasting? Although some studies have shown that prolonged fasting is tolerated in patients with chronic disease and suggested to be well tolerated, it is psychologically uncomfortable for many patients [7,32]. Based on these studies, it is logical to propose that a tRD and/or intermittent fasting might achieve similar goals compared to calorie/dietary restriction but with less side-effects and as such, be more useful in clinical cancer treatment, whereas prolonged fasting may be more effective for cancer prevention.

The beneficial effects of fasting have been demonstrated for many years and throughout the normal course of an organism’s life, fluctuations in available nutrients are expected, and periods of starvation are common [1,2,33]. Adaptive responses have developed over the organism’s evolution to protect it from potentially fatal dangers during these periods of starvation from any of multiple potential environmental conditions. Many past and recent studies have shown that starvation-induced stress resistance is evident and conserved in a variety of different species [34–37]. In both yeast and *Escherichia coli*, glucose starvation increases protection against oxidative stress and, in yeast alone, even a significant increase in longevity [33,38–40]. Worms and flies have been shown to benefit from the same increased resistance against oxidative stress after starvation owing to the diversion of energy from cell growth to protection [21,41,42]. Multiple studies also show that fasting protects the rat brain, mouse kidney and liver, and human liver from ischemia injury [43–46]. In addition, fasting or 10–30% decrease in calorie intake increases lifespan up to 50% and prevents carcinogenesis in spontaneous, chemical or radiation-induced tumorigenesis in several mammalian experimental models [47–50]. More important, recent studies further suggest that fasting can crosstalk with sirtuins, a longevity gene family, which have been suggested to be critical in aging and carcinogenesis.

Sirtuins as metabolic sensors of nutrient availability

Sirtuins, the class III histone deacetylase family, share the homology with yeast silent information regulator 2 (Sir2) and use nicotinamide adenine dinucleotide (NAD)+ as a cofactor [51,52]. Humans have seven sirtuins (SIRT1–SIRT7), which differ in their subcellular localization. SIRT1, SIRT6 and SIRT7 are nuclear sirtuins, which regulate several critical transcription factors of many metabolic pathways [53,54]. SIRT2 is located primarily in cytoplasm and is able to regulate cellular mitosis to prevent genomic instability

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[10,13]. SIRT3, SIRT4 and SIRT5 are located in mitochondria. SIRT3 has been demonstrated to be a legitimate tumor suppressor by regulating mitochondrial energy homeostasis, suppressing ROS production via MnSOD deacetylation [14,19,55], whereas SIRT4 functions as a tumor suppressor as well by mediating a DNA damage dependent block in glutamine metabolism [56]. Sirtuins require NAD+ as a cofactor to deacetylate target proteins and modify their function and as such, it has been proposed that sirtuins are at the nexus of cellular energy metabolic regulation which provides a possible link between cellular energy status and metabolic pathways [9,57]. Based on previous work, it is proposed that when cells are in a nutrient-rich environment, they favor glycolysis to produce energy and induce increased NADH levels as well as decreased NAD+ levels, and this results in inactivation of the enzymatic activity of sirtuins. In contrast, when cells are in an environment that lacks sufficient nutrients that could be considered as nutrient starvation or stress, similar to fasting, this could induce NAD+ levels in the nucleus and cytoplasm resulting in increased enzymatic activity of sirtuins [14].

Although sirtuin proteins appearto direct, at least in some significant part, various enzymatic activities including ADP-ribosylation, desuccinylation, demalonylation, depropionylation and debutyrylation, the most important function in metabolism is proposed to be the regulation of deacetylation [14,58]. The nutrient-sensitive sirtuin-mediated post-translational acetyl modifications of diverse protein substrates add another layer of control over metabolic pathways and place sirtuins as critical master regulators in many cellular processes involved in longevity and aging-related diseases [59,60]. Whole body *Sirt1*-null mice and tissue-specific *Sirt1* knockout mice do not display certain behaviors such as improved glucose and insulin metabolism, reduced IGF-1 signaling and mitochondrial autophagy, observed in wild-type mice under calorie restriction. In accordance with these findings, mice overexpressing *Sirt1* exhibit leanness, improved glucose tolerance and decreased blood cholesterol and insulin levels, which are all phenotypes observed in calorie restricted wild-type mice [61–65]. However, the precise role of SIRT1 in fasting is still not fully understood and seems to vary in a tissue specific manner [66,67].

The mitochondrial sirtuins have been suggested to be involved in the response to nutrient availability as well with SIRT3 playing the most significant role. There are several reasons for this. First, mitochondria are the hubs of metabolism and mitochondrial proteins appear to be acetylated at a high frequency, with at least 20% of mitochondrial proteins found to be acetylated [68]. Most of these acetyl modifications affect enzymatic activity, protein interaction and complex stability [12]. Although SIRT3–SIRT5 are all located in mitochondria, SIRT3 has been shown to exclusively deacetylate mitochondrial proteins[15▪▪] as demonstrated by the increase in the acetylation of mitochondrial proteins in *Sirt3*$^{-/-}$ mice, suggesting an important impact in metabolic control [69]. Second, several energy pathways responding to fasting/calorie or dietary restriction including fatty acid oxidation, amino acid metabolism, ketogenesis and tricarboxylic acid (TCA) cycle are confined in mitochondria [70–73]. Third, mitochondrial dysfunction is largely implicated in cancer and, for example, SIRT3-driven metabolic reprogramming may be particularly important in cancer because most cancer cells have altered metabolism compared to normal cells [12,15▪▪,74]. More specific, SIRT3 is activated after calorie/dietary restriction, which results in suppressed production of ROS and enhanced oxidation of fatty acids, providing a

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link between calorie/dietary restriction and metabolic adaptations [75–77]. Loss of SIRT3 triggers oxidative damage, ROS-mediated signaling, and metabolic reprogramming to support proliferation and tumorigenesis. Thus SIRT3 can be considered as a master regulator of metabolic pathways to promote metabolic homeostasis in response to diverse nutrient signals [16,58].

Fasting, sirtuins and cancer prevention

Cancer is a disease of metabolism that has been closely linked to aging and oxidative damage [78–82]. More significantly, research has turned toward the potential of fasting to prevent cancer development, retard tumor growth and improve the efficacy of chemotherapy treatments [7]. Recent studies suggest that sirtuins may play an important role in fasting-associated benefits by regulating the cellular metabolism that directs a more efficient energy production phenotype that is also associated with a decrease in the production of damaging ROS. In this regard, it is reasonable to ask whether sirtuins are involved in the reduction of cancer incidence observed after calorie restriction or fasting. For example, SIRT3 functions as a tumor suppressor as evidenced by the development of mammary tumors in *Sirt3* knockout mice [12]. One of the major proposed tumor suppression mechanisms of SIRT3 is by regulating cellular metabolic homeostatic poise [13,83,84]. In this regard, it has been shown that SIRT3 increases MnSOD activity and decreases intra-cellular superoxide level by deacetylating lysines 122 and 68 [11,12,55]. Someya *et al.* [75] also showed that SIRT3 directly deacetylates IDH2, promoting oxidative decarboxylation, which in turn increases NADPH levels and the abundance of active glutathione. In addition to SIRT3-induced decreased ROS production, HIF1α is destabilized, which further inhibits tumorigenic capacity [15▪▪]. Based on these results, it is reasonable to suggest that one possible mechanism related to the tumor suppressive effect of calorie restriction and fasting could be the activation of SIRT3; however, there are no laboratory findings to support this hypothesis.

Furthermore, in the context of cancer, it is well known that the increased glycolysis may be used by cancer cells for macromolecular biosynthesis [85–87]. However, upon nutrient starvation, cells should redistribute the limited resource to maintain survival and shut down unnecessary energy expenditures, such as growth, synthesis and reproduction. As such, it seems reasonable to suggest that the cell would improve or increase the efficiency of the mechanisms that produce energy. In this regard, SIRT3 directs proteins regulating both oxidative phosphorylation and TCA cycle, which are critical pathways that determine the method of mitochondrial energy production. For example, it has been published that the subunits of complex 1–2 as well as succinate dehydrogenase activity are regulated via SIRT3-dependent deacetylation [88–90]. The deacetylation of these proteins would ensure oxidative phosphorylation complex assembly and subunit interaction to coordinate fuel oxidation into TCA cycle and substrate delivery to oxidative phosphorylation. Thus, it is possible that SIRT3 activation after fasting would redirect cellular energy production from glycolysis to oxidative phosphorylation and TCA cycle, which might inhibit the macromolecular biosynthesis pathway and slow down tumor cell proliferation.

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Fasting in cancer therapy

Many chemotherapy drugs are believed to cause tumor cell death through genotoxicity from oxidative stress [91]. Therefore, it is suggested that the benefits of nutrient interventions seen in oxidative stress resistance may extend from cancer prevention to therapeutic intervention [92,93]. A series of recent studies by Longo *et al.* have shown that fasting can selectively protect healthy cells while sensitizing tumor cells to drug treatment. Wild-type yeast cells compared with yeast cells expressing an oncogene-like form of Ras were found to be more resistant to hydrogen peroxide or menadione-induced damage after starvation, both of which mimic the action of chemotherapy drugs, whereas the same results were observed in short-term starved mice, which seemed to show enhanced resistance against etoposide [92,93].

Interestingly, it has been demonstrated that starvation not only protected normal cells but also sensitized cancer cells to chemotherapy [8▪▪]. In particular, 4T1 cells treated with either serum from fasted mice or serum with decreased levels of glucose and insulin-like growth factor 1 (IGF-1) mimicking fasting conditions, showed increased sensitivity to chemotherapy drugs doxorubicin and cyclophosphamide. The restriction in glucose and IGF-1 also retarded tumor proliferation and sensitized cells to doxorubicin and cyclophosphamide in 15 other mice and human cancer cell lines including melanoma, glioma and neuroblastoma among others. In-vivo experiments confirmed the ability of fasting to retard tumor growth and sensitize cancer cells to drug treatments. In mice bearing subcutaneous allografts of several murine and human cancer cell lines, two cycles of fasting, complete food starvation for 48–60 h, were able to produce the same retardation of tumor growth as two cycles of cyclophosphamide or doxorubicin treatment. Even more significantly, when fasting is coupled with chemotherapy treatment, tumor size was maintained at less than half of that in mice undergoing drug treatment alone [8▪▪]. These results suggest that fasting has a great potential to benefit the treatment of a wide variety of cancer types.

The mechanism behind the selective protection of normal cells and the sensitization of cancer cells to chemotherapy after fasting remains largely unknown, although previous studies have shown that this phenomenon may be attributed to the reduced levels of IGF-1 and many of its downstream effectors in nutrient signaling pathways such as Akt, Ras, mTOR and FoxO. It is possible that as growth factors and proliferation signals drop during fasting, normal cells switch from growth to a maintenance phase focused on repair and conservation of resources [93,94]. Again, taking under consideration that sirtuins function as metabolic sensors of nutrient availability, it would be interesting to check whether sirtuins are implicated in the response of either normal cells and/or cancer cells to chemotherapy. In this regard, the enhanced activity of sirtuins after fasting may force normal cells to turn off the metabolic pathways required for proliferation and shift them to a more efficient ATP production pathway resulting in a chemoresistant phenotype. For example, SIRT3-induced increase of MnSOD and/or IDH2 activity may further help normal cells to alleviate oxidative stress after chemotherapy [11,18,55,75]. However, it is possible that cancer cells are more sensitive because of the inability to activate sirtuins rendering them unable to adapt to a variety of extreme environments including starvation. Alternatively, another scenario

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could be that sirtuins have different partners in cancer cells as compared with normal cells, which results in the activation of different cellular responses leading to either a chemoresistant or chemosensitive phenotype.

CONCLUSION

There is rapidly growing interest in the biology of sirtuins and its connection to carcinogenesis as well as other age-related diseases. Sirtuins have been suggested to relate to distinct and critical functions in maintaining metabolic homeostasis. The relationship between nutrient starvation, particularly fasting, and cancer therapy has attracted significant interest. This dietary intervention has been shown to be beneficial in many diseases including aging and cancer. More important, the fasting-affected pathways share common targets with sirtuins. Although our mechanistic understanding of this coordination is incomplete, it is clear that the crosstalk between sirtuins and fasting may provide us with a new rationale that may further open up new opportunities for therapeutic interventions in a broad range of metabolic and degenerative diseases.

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KEY POINTS

- Fasting in mammals could sensitize cancer cells but not normal cells to chemotherapy, thus inducing selective killing.
- The mechanism of fasting-induced cancer cell preferential killing may be attributed to the failure to induce a stress maintenance response by shifting the cellular metabolism toward energy conservation and damage repair as opposed to normal cells.
- Sirtuins have been demonstrated as nutrient sensors and can respond to cellular nutrient availability by directly regulating the cell energy homeostasis and cellular defense pathways.
- SIRT3 has been demonstrated to play an important role in cell metabolism regulation, cellular redox balance, and tumor suppression.
- SIRT3 shares the most common targets of fasting activated signaling pathways suggesting that SIRT3 could act as a master metabolic regulator in fasting-induced beneficial effects on aging and carcinogenesis.