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## Calorie restriction and cancer prevention: metabolic and molecular mechanisms

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### Abstract

An important discovery of recent years has been that lifestyle and environmental factors affect cancer initiation, promotion and progression, suggesting that many malignancies are preventable. Epidemiological studies strongly suggest that excessive adiposity, decreased physical activity, and unhealthy diets are key players in the pathogenesis and prognosis of many common cancers. In addition, calorie restriction (CR), without malnutrition, has been shown to be broadly effective in cancer-prevention in laboratory strains of rodents. Adult-onset moderate CR also reduces cancer incidence by 50% in monkeys. Whether the anti-tumorigenic effects of CR will apply to humans is unknown, but CR results in a consistent reduction in circulating levels of growth factors, anabolic hormones, inflammatory cytokines and oxidative stress markers associated with various malignancies. Here, we discuss the link between nutritional interventions and cancer prevention with focus on the mechanisms that may be responsible for these effects in simple systems and mammals with a view to developing chemoprevention agents.

### INTRODUCTION

Cancer is a complex multistage disease associated with accumulation of multiple DNA mutations that cause a deregulation of cell proliferation and differentiation, loss of normal tissue organization, and eventually tissue invasion and dislocation to distant sites (1). DNA damage, which occurs continuously in both the dividing and non-dividing cells of the human body, and can increase after exposure to exogenous genotoxic carcinogens (e.g. radiations, chemicals, tobacco smoke, viruses, aflatoxin and other food derived carcinogens), can be prevented or repaired by endogenous protective small molecules and enzymes (2–4). However, these detoxification and repair systems may fail, especially in environments that promote cell proliferation and inhibit cell apoptosis, but also in non-dividing cells in which the opportunity for repair may be limited (5,6). Accumulation of multiple DNA mutations in critical genes (i.e. oncogenes or tumor suppressor genes) of particular cells, if not properly controlled through

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induction of senescence or apoptosis, can lead to uncontrolled cell proliferation and progressive transformation of normal human cells into highly malignant tumor cells (5–7). Moreover, recent data suggest that the surrounding microenvironment and cell-to-cell interactions between cancer cells and their neighbour stromal and inflammatory cells play a central role in driving tumor cell proliferation, tissue invasion and metastasis, that ultimately are responsible for ~90% of human cancer deaths (8,9).

Metabolic, hormonal and growth factor alterations associated with increased food consumption, decreased physical activity, and excessive adiposity, affect the regulation and expression of genes involved in DNA repair, cell proliferation and differentiation or apoptosis, allowing cells to accumulate damage and mutations, survive, proliferate and under permissive conditions, undergo malignant transformation (10,11). These detrimental effects can be potentiated by exposure to non-genotoxic carcinogens (e.g. ethanol, saccharin, 1,4-dichlorobenzene, 17beta-estradiol, arsenic and beryllium) that induce cell damage, by inflammation, increased oxidative stress and secretion of anabolic hormones, immunosuppression, and activation of signal-transduction pathways that result in genomic instability, loss of proliferation control, and resistance to apoptosis (12).

Calorie restriction (CR) without malnutrition is the most potent and reproducible physiological intervention for increasing lifespan and protecting against cancer in mammals (13,14). CR reduces the levels of a number of anabolic hormones, growth factors and inflammatory cytokines, reduces oxidative stress and cell proliferation, enhances autophagy and several DNA repair processes (13,14). Hence, understanding the metabolic and molecular mechanisms responsible for the CR-mediated cancer preventive effect has the potential to lead to drugs and therapies for broad-spectrum prevention and treatment of cancer. Here, we discuss nutritional interventions that have been shown to prevent cancer and to ameliorate cancer prognosis. We also describe the genetic pathways and mechanisms that appear to be crucial for the effects of CR and discuss the evidence for potentially protective but also detrimental effects in humans.

## NUTRITION AND CANCER

Epidemiological data on geographical and chronological variations in cancer incidence have shown that environmental factors have profound effects in the initiation, promotion and progression of some of the most common cancers in Western countries (15,16). In fact, studies of populations migrating from a low- to a high-risk area for cancer have shown major changes in rates of several common cancers. For example, the incidence of stomach cancer decreased and the rates of breast, colon and prostate cancer increased within a single generation after migration of Japanese people from Japan to Hawaii (15). Moreover, the recent shift from traditional dietary patterns to Western diet patterns in many developed and developing countries has produced a striking increase in the most common cancers (e.g. colon, breast and prostate cancer) within populations previously considered to have a low prevalence rate (15, 16). For example, in the last 4 decades in Japan the age-standardized incidence rate of colon cancer has increased 9.4 times for males and 4.7 times for females, in conjunction with a significant increase in height and body weight (17). These studies, together with the investigation of lifestyle factors and behaviors, have led to the conclusion that the majority of cancer deaths in many developed countries can be attributed to factors such as unhealthy diets, tobacco, alcoholism, infections and occupational exposures.

In particular, data from several epidemiological studies support the theory that diet plays an important role in the initiation, promotion and progression of many common cancers in Western countries (18). Despite frequent reports of specific vitamins, micronutrients, nutrients, or foods having a favorable or harmful effect on cancer risk, few of these have a recognized cause-effect link to cancer (i.e. fried, broiled or roasted red meat, aflatoxin-contaminated food, preserved

salty foods, excessive alcohol consumption) (19,20). These results are often based on data obtained from cell culture or animals studies, and not from randomized clinical trial in humans (19). To date, there is consensus that excessive adiposity due to overconsumption of energy-dense foods and a sedentary lifestyle increases the risk of developing cancer (10,21). Nonetheless, data from several epidemiological studies suggest that cancer risk can also be reduced by an overall dietary pattern that favors a high intake of plant foods (e.g. vegetables, beans, fruits, and whole grains) rich in a wide range of phytochemicals, and a limited consumption of animal fat, meat and dairy products (18,21,22). Greater consumption of a wide variety of vegetables, beans and fruits has been associated with a lower risk of developing colon, lung, oral, esophageal, and stomach cancer (22). Evidence that high intake of vegetables and fruits reduce the risk of developing breast and prostate cancer is less strong (22).

### **Excessive adiposity and cancer risk**

Several epidemiological studies have consistently shown associations between adiposity and increased risk of cancers of the endometrium, breast (postmenopausal), colon, esophagus (adenocarcinoma), kidney (renal-cell), pancreas, gallbladder and liver (10,23). No association is seen between adiposity and the risk of developing lung and prostate cancer, whereas premenopausal breast cancer is inversely correlated with body mass index (10,21).

Excessive adiposity due to chronic energy imbalance is associated with increased oxidative stress, insulin resistance, inflammation, and changes in hormone and growth factor concentrations that play a key role in the pathogenesis of many cancers (Figure 1) (10,21,23, 24). Persistent positive energy balance promotes hypertrophy of adipose tissue, reduced adiponectin production, insulin resistance, and compensatory hyperinsulinemia (25). Adiposity generally shows a direct linear relationship with serum insulin concentrations (26). Chronically elevated insulin concentrations have been associated with cancers of the breast, colon, pancreas, endometrium (27–30). A number of mechanisms likely account for the tumorigenic effects of insulin, which exerts direct powerful mitogenic effects through the insulin receptor expressed on many cell types, especially in pre-neoplastic cells (31). In addition, hyperinsulinemia inhibits the hepatic production of sex hormone-binding globulin and therefore increases the circulating concentrations of bioavailable sex hormones (32). Hyperinsulinemia also stimulates the ovarian production of androgens (33). Finally, insulin increases the biological activity of IGF-1, in part by reducing synthesis and secretion of IGFBP-1 (34). Adipose tissue is considered to be one of the major sources of extra-glandular estrogen, produced by aromatization of androgen precursors (35). Estrogens, androgens and IGF-1 are strong mitogens for cells, and stimulate the development and growth of several tumors (31,35,36). For example, women who are overweight, especially those with visceral obesity, which is associated with increased risk of breast cancer in postmenopausal women, frequently have insulin resistance, hyperinsulinemia, low plasma levels of sex hormone-binding globulin, and high total and free sex hormone levels (37). Finally, fat cells produce and secrete molecules such as IGF-1, IL-6, leptin and type VI collagen that promote cell survival and tumor growth (25,38,39).

Recent data indicate that long-term cancer mortality after weight loss induced by gastric bypass surgery is significantly reduced (40,41). Exercise- and/or CR-induced weight loss improves several metabolic and hormonal alterations associated with excessive adiposity in overweight and obese subjects (13,42). Weight loss induced by a negative energy balance is associated with decreased fat cell size, a reduction in visceral, hepatic and skeletal muscle fat, improved adipokine profile and insulin sensitivity, and reduced circulating insulin levels (25,43–45). Reduced fat mass is also associated with a reduction in circulating estrogens levels due to reduction in aromatase activity (23,33,46). The reduction in insulin levels is associated with: (1) increased SHBG and reduced free estrogens and testosterone, (2) increased IGFBP-1 and

reduced free IGF-1 (10,21,23,32,34,37,42). Weight loss is also associated with a reduction in inflammatory cytokines and prostaglandins, and in several markers of oxidative stress and DNA damage (47–50). Finally, a 13 months weight loss intervention has recently been reported to be associated with increased telomere length in the rectal tissue biopsies obtained from a small group of obese patients (51), suggesting that CR may contribute to the prevention of telomere shortening, which may also be important in modulating the pathogenesis of cancer and biology of aging (52).

### **Calorie restriction and cancer**

In 1909, Moreschi published the first scientific paper to report that CR inhibits the growth of tumors transplanted into mice (53). Subsequently, data have shown that CR, defined as a reduction in calorie intake below usual ad libitum intake without malnutrition, inhibits spontaneous, chemically-induced and radiation-induced tumors in several animal models of cancer (54–60). More recently, adult-onset moderate 30% CR has been shown to reduce cancer morbidity and mortality also in non-human primates. The incidence of cancer (mainly gastrointestinal adenocarcinoma) was reduced by 50% in the CR monkeys as compared to that in controls (61). The age when CR is started, the severity of CR, and the strain/genetic background of the animals determine the magnitude of cancer prevention or delay (54–60, 62). In rodents, 15%–53% reduction in calorie intake below usual ad libitum intake caused a proportionate linear 20%–62% reduction in tumor incidence (63). Nonetheless, the effects of CR on cancer are not homogeneous. Some cancers show a greater response to CR than others, and a small portion of tumors is resistant to the effects of CR (54,56,57,60,62).

The mechanisms responsible for CR-mediated beneficial effects on cancer observed in rodents, and now also in monkeys, which will be discussed in more detail in the following sections, are thought to involve the metabolic adaptations to CR itself (Figure 2), including: 1) decreased production of growth factors and anabolic hormones (64–67); 2) decreased reactive oxygen species production and modulation of the endogenous antioxidant systems, which decrease oxidative stress and free radical-induced DNA damage (68–70); 3) decreased plasma concentrations of inflammatory cytokines and an increase in circulating corticosteroids, ghrelin and adiponectin that results in a reduction in inflammation (71–75), 4) protection against aging-associated deterioration in immunosurveillance (76–77). In addition, CR simultaneously affects multiple processes that are involved in the pathogenesis of cancer, including enhanced DNA repair processes, increased removal of damaged cells through apoptosis, enhanced autophagy and protection from the damaging effects of a range of damaging agents (e.g., toxic and genotoxic compounds) (78–81). Many of the effects of CR are likely mediated by regulating gene expression including the up-regulation of tumor-suppressor genes, of genes promoting DNA and cellular repair, protein turnover, stress resistance, and anti-oxidant genes, and the down-regulation of pro-inflammatory genes and modulation of energy metabolism pathways (82,83). Whether CR with adequate nutrition will reduce cancer incidence in humans is unknown, but data from studies of long-term CR in humans suggest that the metabolic and physiological responses to CR are similar to those in rodents and monkeys (13,84–87).

### **Endocrine regulation of cancer by insulin-like signals**

Insulin-like growth factor 1 (IGF-1), a growth factor produced primarily from the liver, acts synergistically with other anabolic hormones (e.g. insulin, sex steroids), and in relation to calorie and protein availability, to regulate energy metabolism, cell proliferation, cell differentiation, body size and longevity (31,35,36). In addition, IGF-1 exerts a potent mitogenic effect on a variety of cancer cells, by increasing proliferation rate and inhibiting apoptosis (31,88).

Long-term calorie restriction, but not endurance exercise, decreases serum IGF-1 concentration by approximately 30–40% in rodents, and this CR-mediated reduction in IGF-1 levels is believed to play a key role in protecting against cancer and slowing aging (11,64,89–90). The powerful CR-mediated protective effect against carcinogenesis in rodents is reversed by infusing growth hormone or IGF-1 (91–93), further underlining the critical role of these growth factors in the pathogenesis of cancer. In addition, growth hormone receptor (GHR) knockout mice, that have low serum IGF-1 concentrations, exhibit a ~50% reduction in tumor burden and incidence of lethal cancers, in agreement with results in growth hormone (GH) deficient Snell dwarf, in which cancer is either reduced or postponed (94,95). A lower mutation rate in middle-aged GH deficient Ames dwarf mice was observed in kidney, liver and intestine, providing some mechanistic explanation for the delay in neoplastic diseases (96). In contrast, GH overexpressing mice have very high concentrations of IGF-1, increased body size up to 100%, increased incidence and early on-set of tumors, and a marked reduction in lifespan compared to their normal siblings (97). Nonetheless, down-regulation of IGF-I signalling may account for only part of the effects of CR, GH and GHR deficiencies in protecting against cancer. The CR-mediated increase in corticosterone, for example, may also play an important role in preventing cancer, as adrenalectomy reverses the cancer-protective effects of CR, and glucocorticoid supplementation restores inhibition (98).

The role of IGF-1 in the pathogenesis of some human cancers is supported by epidemiologic studies, which have found that high serum concentrations of IGF-I are associated with increased risk of several common cancers, including those of the prostate, breast, and colon (99). An elevated incidence of tumors is observed also in acromegalic patients, who have elevated IGF-I levels (100). Nutrition is one of the major regulators of circulating IGF-1 levels. Fasting in humans markedly reduces serum IGF-1 concentration into the range observed for GH-deficient patients (101), but long-term severe CR does not reduce circulating IGF-1 levels in middle-aged healthy men and women if protein intake is high (102,103). In contrast, strict vegetarians consuming a moderately restricted protein diet (~0.75 g of protein/kg body weight/day) display significantly lower serum concentrations of total and free IGF-1 (102). Moreover, reducing protein intake in individuals practicing severe CR with high protein intake (~1.65 g of protein/kg body weight/day) results in a 25% reduction in serum IGF-1 (from 194 ng/ml to 152 ng/ml), suggesting that protein intake is a key determinant of circulating IGF-1 levels in humans (102). These findings are in agreement with a series of data from epidemiological studies that show a positive association between protein intake and serum IGF-I concentration in both men and women eating typical Western diets (104,105). In women the association between protein intake and circulating IGF-1 concentrations was stronger than the relationship between calorie intake and IGF-I concentration (104). In men animal and vegetable protein consumption was associated with an increase in serum IGF-1 concentrations, whereas calorie intake was associated with an increase in serum IGF-I concentration only in lean men with a BMI <25 kg/m<sup>2</sup> (105). These data might help to explain, at least in part, the link between the consumption of high animal protein Western diets and the increased incidence of two adiposity-independent common cancers, such as prostate and premenopausal breast cancer (15,16,99,106,107). It is important to note that the recommended daily allowance for protein intake in healthy adults is 0.83 g/kg of body weight/day (108), whereas at least 50% of the US males are eating ≥ 1.34 g of protein/kg of body weight/day, which is 40% or more protein than the RDA recommended intakes, and therefore associated with a positive nitrogen balance (108,109). More studies are necessary to understand the metabolic and clinical implications of a chronic positive nitrogen balance on serum IGF-1 and IGF-BPs concentrations, and on cancer biology, especially in sedentary adults with a positive family history for cancer.

The hypothesis that reduction of GH/IGF-I signaling can modulate/prevent cancer in humans can also be tested by following individuals that have deficiencies in the GH/IGF-I axis. Preliminary data from a unique population of GHR deficient patients (Laron dwarfs), who are

deficient in IGF-1, suggest a protective effect against tumorigenesis (110). However, this report is indicative but no conclusive since the mean age of the Laron dwarfs was much lower than the mean age of the controls. Although animal studies suggest that GH and IGF-1 play an important role in modulating cancer, to determine whether CR and/or GH and/or IGF-1 deficiencies indeed reduce cancer risk in human we must await properly controlled studies of CR and Laron dwarfs or similar populations. Nevertheless, even if GH and/or IGF-1 deficiencies may decrease cancer risk, it is well known that low levels of IGF-1 are associated with increased obesity and heart disease in people who are not restricting calorie intake (111). On the other hand, severe CR (when protein intake is normal) causes a reduction in IGF-1 without leading to obesity, and while concomitantly reducing many of the markers of heart diseases (84). Not surprisingly, GH and IGF-1 deficient dwarf mice become obese at middle age and live approximately 40% longer (112), but when these mice are calorie restricted, they no longer become obese and can live up to 100% longer than wild type and ad lib fed mice (113).

## MOLECULAR TARGETS OF CALORIE RESTRICTION

Because CR extends the life span of all the major model organisms used to study aging, the nutrient-response pathways that regulate aging and age-dependent genomic instability in these organisms can provide insights on the mechanisms linking CR and the reduction in cancer. Although worms and flies are excellent model organisms to study aging, their mostly non-dividing cellular network and rare tumor phenotypes limit their value in studies of the mechanisms of age-dependent tumorigenesis. By contrast, the simple *S. cerevisiae* can provide evidence for the fundamental molecular mechanisms of age-dependent genomic instability whereas mice can be studied to determine whether similar mechanisms apply to mammals. Here, we review the major pathways and mechanisms believed to connect CR and genomic instability and/or cancer.

### Pathways that mediate CR-dependent longevity extension regulate genomic instability and cancer-like phenotypes in *S. cerevisiae*

*S. cerevisiae* has the advantages of being perhaps the simplest and the best characterized model system to study aging. As observed in mammals (114), mutations, which increase with age in *S. cerevisiae* by as much as 10-fold, are reduced by CR (115–117). The deletion of either the TOR/SCH9, the yeast homologues of mammalian target of rapamycin (mTOR), and S6 kinase (S6K) and/or AKT genes, respectively, and the down-regulation of the Ras/adenylyl cyclase (AC)/protein kinase A (PKA) pathway postpone this age-dependent increase in spontaneous mutations (118–120). Notably, the down-regulation of the Tor/Sch9 and the Ras/AC/PKA pathways are required to extend life span in yeast by a mechanism that involves the up-regulation of stress resistance transcription factors described below.

Analogously to the accumulation of somatic mutations playing key roles in cancer development in mammals (114), cancer-like mutant cells that “regrow” under conditions that block the growth of normal cells, are generated within aging *S. cerevisiae* populations (116,117). Such regrowth can be studied in *S. cerevisiae* liquid cultures by monitoring the takeover of the culture by the mutated sub-population that utilizes the limited nutrients available to grow while normal cells are aging and dying (116). The frequency of this cancer-like regrowth phenotype is greatly reduced in CR yeast and yeast with mutations in the Tor/Sch9 and Ras/AC/PKA pathways (117). The reduced frequency of age-dependent mutations in CR cells and cells lacking TOR1/SCH9 (118–120) suggests that oncogene homologs control age-dependent regrowth in part by regulating the generation of the one or multiple mutations necessary for growth under unfavorable conditions. The effect of Tor/Sch9 and Ras/AC/PKA on DNA mutations is due in part to regulation of antioxidant defenses and the generation of oxidants. In fact, the generation of oxidants is elevated and resistance to oxidants and life span are decreased in *S.*

*Cerevisiae* expressing an oncogene-like constitutively active RAS2<sup>val19</sup> (121,122) or overexpressing SCH9. Furthermore, CR cells and cells lacking RAS2 or TOR1/SCH9 are highly protected against oxidative damage (119,120,121,123) and age-dependent regrowth frequency doubles in cells lacking cytosolic superoxide dismutase but is reduced in cells overexpressing antioxidant enzymes (116,119,121). Supporting this model is the very high frequency of nuclear mutations in aging yeast lacking mitochondrial superoxide dismutase (SOD)-2 or SOD-1 (115,116), with cells lacking SOD1 being among the genetic manipulations causing the highest rate of DNA mutations. The down-regulation of superoxide generation and increase in antioxidant defenses in Ras/AC/PKA and/or Tor/Sch9 deficient cells is mediated by serine threonine kinase Rim15 and transcription factors Msn2/4 and Gis1, which regulate a number of stress resistance genes including the mitochondrial superoxide dismutase SOD2 (119,122). CR is likely to regulate pro- and anti-oxidant systems by similar mechanisms since life span extension caused by CR is largely reversed in cells lacking these 3 stress resistance transcription factors (i.e. Msn2/4 and Gis1) (124).

In addition to the oxidative stress systems, the Tor/Sch9 and Ras/AC/PKA pathways regulate the expression of a number of DNA repair genes (125). Among them is the REV1 gene, which functions in the error-prone translation repair system together with the polymerase Polzeta. It is the Rev1/Polzeta system that is responsible for a major portion of the age-dependent DNA mutations (119). The evidence suggests that the Tor/Sch9 pathway promotes superoxide generation and reduces the anti-oxidant protection by down-regulating Msn2/4 and Gis1. These changes result in increased oxidative damage to the DNA but also in increased expression of the REV1 gene, which, together, promote age-dependent point mutations (119). The activation of the error prone Rev1/Polzeta appears to generate point mutations as part of a process required to prevent the potentially more detrimental gross chromosomal rearrangements that occur when replication is stalled. Whether Rev1/Polzeta is involved in the CR-dependent effect on DNA protection is unknown.

### **Molecular pathways that mediate the anti-cancer effects of calorie restriction**

Studies in yeast, worms, and flies indicate that the insulin and IGF-I pathways may be important for aging and cancer in mammals (Figure 3). In mice, mutations in the Prop-1 or Pit-1 genes, which cause severe deficiencies in GH and IGF-I, extend life span by 25 to 65% and cause dwarfism (126,127). These deficiencies appear to mediate the effects of Prop-1 and Pit-1 mutations on longevity, since the mice that cannot release GH in response to growth hormone releasing hormone also live longer (128). In fact, dwarf mice with high plasma GH, but a 90% lower IGF-I (GHR/BP null mice) and heterozygous female IGF-I receptor knockout mice live longer than the wild type controls (129,130).

Reduced insulin/IGF-1 signaling in mice not only increases life span, but also delays or reduces aging-related pathology, most notably spontaneous tumors (94,131) in agreement with the association between IGF-1 level and cancer incidence. Notably, IGF-I and age-dependent cancer are both decreased in CR mice (14,54–60). The mechanism behind the increased survival and reduced cancer incidence associated with CR and low IGF-1 signals may be partly explained by the increased resistance to oxidative and other types of damage demonstrated in yeast, nematode and flies with defects in insulin/IGF-1-like pathways (132). In fact, fibroblasts from long-lived, adult Ames or Snell dwarf mice as well as growth hormone receptor knockout mice are resistant, *in vitro*, to a variety of toxic agents, such as hydrogen peroxide and ultraviolet light (133) and hepatocytes from Ames dwarf mice more readily undergo apoptosis than wild-type cells, when experiencing an oxidative challenge (134) and IGF-I reverses the beneficial effects of calorie restriction against a bladder carcinogen (93). Activation of the transcription factors NF-E2-related factor 2 (Nrf2), which increases the transcription and activity of a variety of antioxidative and carcinogen-detoxification enzymes, may also be important in mediating

the anti-cancer effects, but not the insulin sensitizing and anti-aging effects of CR. The anti-tumorigenesis effects of CR are significantly impaired in Nrf2 deficient mice exposed to carcinogens (135). In agreement with these results, Mn-superoxide dismutase (MnSOD) heterozygous knockout mice, that have reduced MnSOD activity, have increased levels of oxidative damage to DNA in all tissues, a 100% increase in cancer incidence, but average and maximal lifespan is not affected (136). These data suggest that the relationship between oxidative stress, cancer and aging is complex, and may also indicate that the conditions that increase mutations and cancer are not necessarily accelerating aging.

In addition to reducing growth and enhancing apoptotic pathways, CR and reduced IGF-I may contribute to cancer by also reducing genomic instability, possibly via a Ras- or phosphatidylinositol-3 kinase (PI3K)/Akt/Tor/S6kinase-dependent mechanisms. For example, the degree of activation of PI3K in cancer cells plays an important role in regulating cell proliferation, tumor growth, and sensitivity to CR. Tumors that are CR-resistant originate from cancer cells with constitutive activation of the PI3K pathway, which in culture proliferate in the absence of IGF-1 and insulin (137). Replacement of an activated mutant allele of PI3K with a wild-type PI3K allele in these cancer cells restored CR-sensitivity in vitro and in vivo, underlining the importance of the insulin/IGF-1 signalling pathway in mediating the anti-cancer effects of CR. The expression of the tumor suppressor phosphatase and tensin homologue (PTEN), an inhibitor of PI3K activity, in the CR-resistant cancer cells was also sufficient to restore CR-sensitivity in vitro and in vivo in tumor xenografts animal models (137). Several downstream effectors of PI3K/AKT [e.g. forkhead transcription factors (FOXO), mTOR, adenosine monophosphate (AMP)-activated protein kinase (AMPK), S6K, silent mating type information regulation 2 homolog 1 (SIRT1)] might be responsible for the anti-tumorigenic effects of CR (138–142). Interestingly, although the nuclear transcription factor p53, a tumor suppressor that regulates cell cycle, apoptosis and cell senescence, down-regulates the expression of the IGF-1 receptor and the insulin/IGF-1 pathway in post-mitotic fully differentiated cells (143), the anti-tumorigenic effects of CR are similar in p53 overexpressing and p53 knockout mice (144). A better characterization of the molecular signaling pathways by which CR mediates its cancer inhibitory activity in some, but not all cancers, is essential to design new drugs and interventions that prevent tumor initiation or block tumor promotion and progression.

### Conclusions and future directions

The probability of developing cancer is remarkably high in the US, with approximately 44% of the men and ~37% of the women will develop cancer during their lifetime (145). Although genetic inheritance influences the risk of cancer (146), most of the variation in cancer risk across populations and among individuals is due to lifestyle and environmental factors. Data from experimental and epidemiological studies indicate that excessive adiposity due to excessive energy intake and minimal physical activity increase the risk of developing cancer. In contrast, calorie restriction without malnutrition, and possibly protein restriction, prevent cancer. More studies are needed to elucidate the molecular mechanisms underlying the beneficial effects of CR and other interventions (e.g. fasting, protein restriction, phytochemicals) in preventing cancer by avoiding accumulation of DNA damage or by potentiating the regression of preneoplastic lesions. More research is also needed to understand how we can block cancer before it becomes invasive or metastatic, since metastases are the cause of 90% of human cancer deaths (9). Cancer is not a disease limited to a number of proliferating mutated cells, but a complex process that also involves interactions with the neighboring not-mutated mesenchymal and inflammatory cells that are also affected by aging and/or cancer risk factors (8,9). CR (and other interventions) by reducing the activity of pro-aging pathways, reducing growth and inflammation in the pre-cancerous and normal neighbor cells, and increasing apoptosis in damaged cells may reduce oncogene mutations frequency but also



modulate the growth and invasiveness potentials of the mutated cancerous cells. Hence, understanding the role of CR and of other dietary manipulations in cancer, and identifying the metabolic and molecular mechanisms responsible for the CR-dependent cancer preventive effect has the potential to lead to drugs and therapies for broad-spectrum prevention and treatment of cancer.

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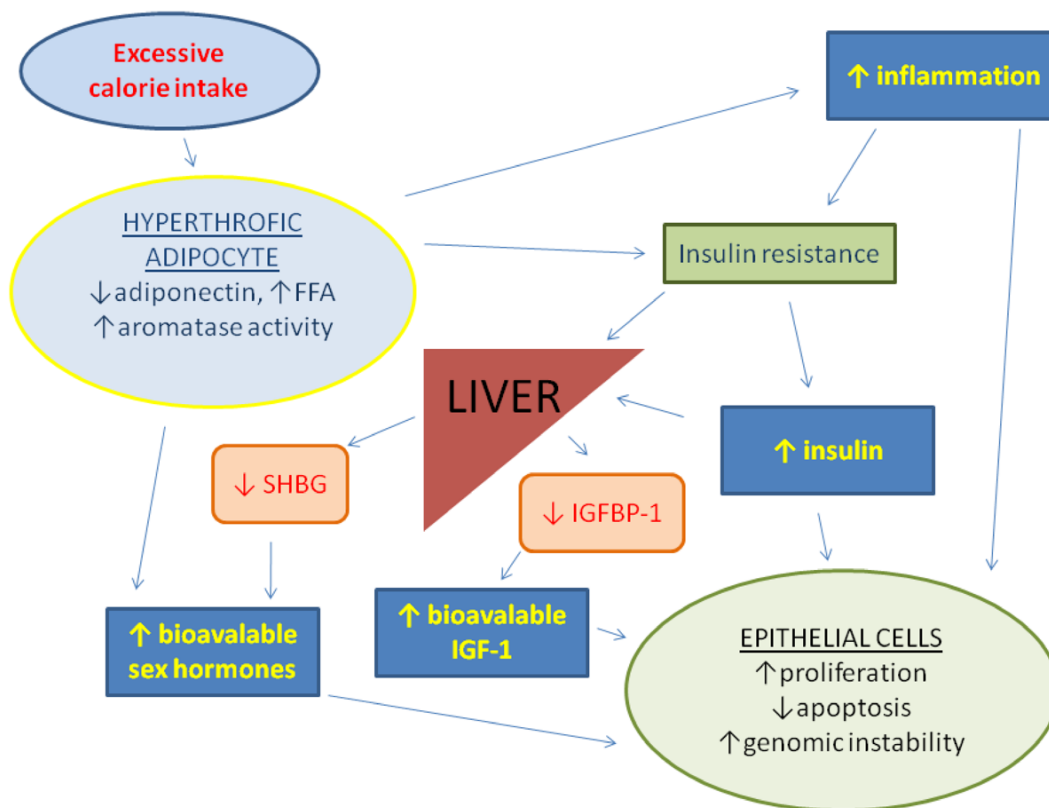
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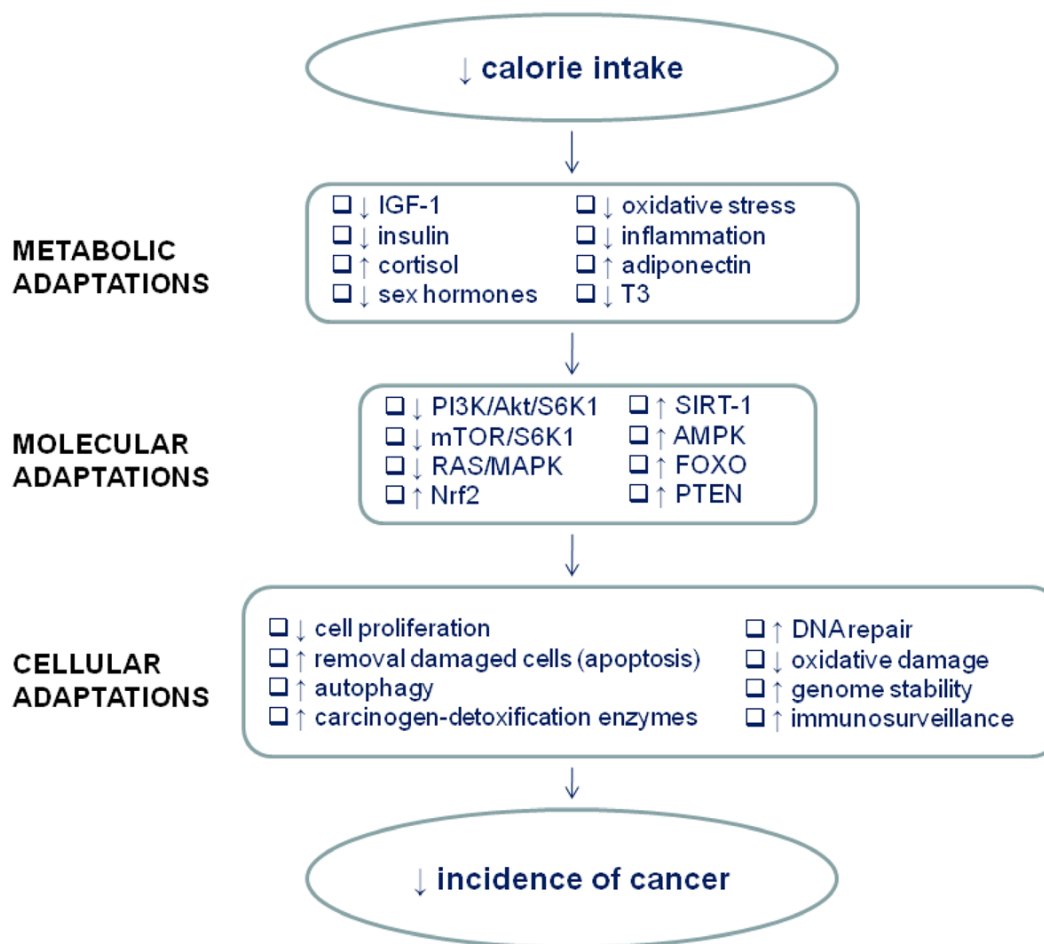
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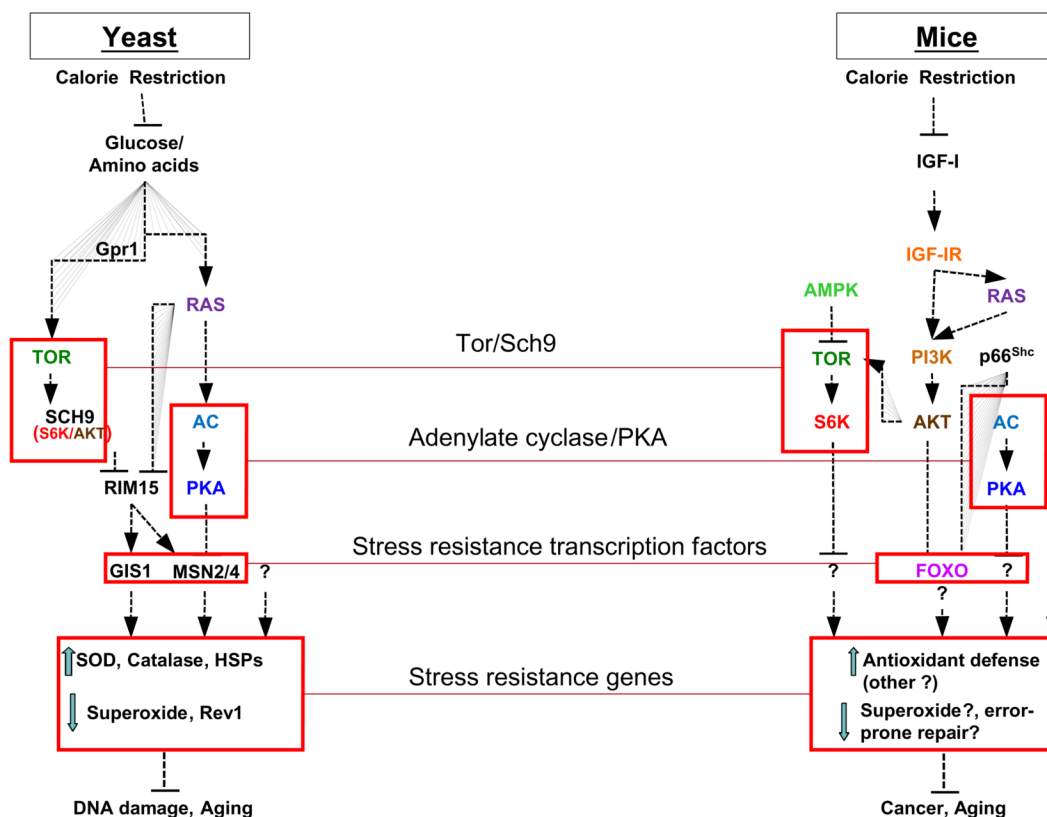
**Fig. 1. Effects of excessive calorie intake and adiposity on hormones and growth-factor production and cell proliferation**

Excessive calorie intake and a sedentary lifestyle promote hypertrophy of adipose tissue, reduce adiponectin production, and increase circulating free fatty acids (FFA) and inflammation, leading to insulin resistance and compensatory hyperinsulinemia. Increased serum insulin concentration causes a reduction in hepatic synthesis of insulin-like growth factor binding protein 1 (IGFBP1) and steroid hormone binding globulin (SHBG), that leads to increased bioavailability of insulin growth factor 1 (IGF-1) and sex hormones. Adipose tissue is also a major source of extra-glandular estrogens. Chronically elevated circulating levels of insulin, IGF-1, sex hormones and inflammatory cytokines promote cellular proliferation, genomic instability, and inhibit apoptosis in many cell types.



**Fig. 2. Mechanisms for cancer prevention by calorie restriction**

CR causes several key metabolic/hormonal adaptations, that alter the expression of several genes and signaling pathways (up-regulation of certain genes/signaling pathways and down-regulation of others as indicated by the arrows), which produce major cellular adaptations (e.g. a reduction in cell proliferation, increased removal of damaged organelles or cells via autophagy or apoptosis, up-regulation of DNA repair systems and genomic stability) that result in a reduced cancer incidence (see the text). T3 = triiodothyronine; PI3K = phosphatidylinositol-3 kinase; AKT = kinase AKT, also known as protein kinase B; S6K1 = ribosomal S6 protein kinase 1; mTOR = mammalian target of rapamycin; MAPK = mitogen-activated protein kinase; NRF2 = transcription factors NF-E2-related factor 2; SIRT-1 = silent mating type information regulation 2 homolog 1; AMPK = adenosine monophosphate (AMP)-activated protein kinase; FOXO = Forkhead transcription factors; PTEN = phosphatase and tensin homolog.



**Fig. 3. Pro-aging and pro-cancer pathways in yeast and mice**

Similar pathways, including Ras, Tor, S6 kinase (S6K), adenylate cyclase (AC), and PKA, have been shown to promote aging in both yeast and mice. In yeast, CR causes the down-regulation of these proteins, which promote DNA mutations by reducing the activity of stress resistance transcription factors including Msn2/4 and Gis1 and subsequently increasing the level of superoxide and the activity of error-prone polymerases (Rev1, etc). In yeast, this DNA damage-promoting mode can occur largely independently of cell division. In mice, orthologues of yeast Tor, S6K, AC, and PKA promote aging but are also components of some of the most common oncogenic pathways. CR reduces IGF-I and consequently can reduce the activity of protein functioning downstream of IGF-IR including Tor and S6K. Activation of Tor and S6K but also of AC and PKA may promote DNA damage and cancer in part by promoting cell growth and inhibiting apoptosis in damaged cells and in part by promoting aging and genomic instability independently of the rate of cell growth. These pathways may also contribute to cancer and metastases by affecting inflammation and the cellular environment of the malignant and pre-cancerous cells. The mechanisms connecting IGF-I signaling pathways and cancer in mammals are poorly understood but may involve mechanisms similar to those identified in yeast (147).