Effects of Dietary Restriction on Cancer Development and Progression

Daniele Fanale, Rossella Maragliano, Alessandro Perez, and Antonio Russo

Abstract

The effects of caloric restriction on tumor growth and progression are known for over a century. Indeed, fasting has been practiced for millennia, but just recently has emerged the protective role that it may exert toward cells. Fasting cycles are able to reprogram the cellular metabolism, by inducing protection against oxidative stress and prolonging cellular longevity. The reduction of calorie intake as well as short- or long-term fasting has been shown to protect against chronic and degenerative diseases, such as diabetes, cardiovascular pathologies, and cancer. In vitro and in vivo preclinical models showed that different restriction dietary regimens may be effective against cancer onset and progression, by enhancing therapy response and reducing its toxic side effects. Fasting-mediated beneficial effects seem to be due to the reduction of inflammatory response and down-regulation of nutrient-related signaling pathways able to modulate cell proliferation and apoptosis. In this chapter, we will discuss the most significant studies present in literature regarding the molecular mechanisms by which dietary restriction may contribute to prevent cancer onset, reduce its progression, and positively affect the response to the treatments.

Keywords

Caloric restriction • Cancer • Cell proliferation • Diet • Dietary restriction • Fasting • Feeding • IGF-1 • Inflammatory response • Long-term starvation • Molecular pathways • Oxidative stress • Short-term starvation • Therapy response

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List of Abbreviations

AKT	Serine/threonine kinase
AMPK	AMP-activated protein kinase
APN	Aminopeptidase N
ATM	Ataxia telangiectasia mutated
BER	Base excision repair
Bm1	B Lymphoma Mo-MLV insertion region 1 homolog
C/EBPβ	CCAAT/enhancer-binding protein β
CAAs	Cancer-associated adipocytes
ChK2	Checkpoint kinase 2
CR	Caloric restriction
DEN	Diethylnitrosamine
DR	Dietary restriction
DSBs	Double-strand breaks
DSR	Differential stress resistance
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinase
FGF21	Fibroblast growth factor 21
FOS	FBJ murine osteosarcoma viral oncogene homolog
FOXO	Forkhead box subgroup O
GCN2	General control nonderepressible 2
H2AX	H2A histone family member X
HCC	Hepatocellular carcinoma
HDAC1	Histone deacetylase 1
HER2	Human epidermal growth factor receptor 2
HopX	HOP homeobox
HSL	Hormone-sensitive lipase
IGF-1	Insulin-like growth factor 1
IGFBP-1	IGF-binding protein 1
IL-6	Interleukin 6
KD	Ketogenic diet
Lgr5	Leucine-rich repeat containing G-protein-coupled receptor 5
LTS	Long-term starvation
MAPK	Mitogen-activated protein kinase.
Msn2/4	Moesin 2/4
mTOR	Mammalian target of rapamycin
mTORC1	mTOR complex 1
NF-KB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NSCLC	Nonsmall cell lung cancer
OGG1	8-Oxoguanine DNA glycosylase 1
PARP-1	Poly (ADP-ribose) polymerase 1
PI3K	Phosphoinositide 3-kinase
PKA	Protein kinase A
PSA	Prostate-specific antigen

RAF	V-Raf-1 murine leukemia viral oncogene homolog
Ras	Rat sarcoma viral oncogene homolog
REV1	DNA-directed polymerase
ROS	Reactive oxygen species
SIRT1	Sirtuin 1
SOD2	Superoxide dismutase 2
SSBs	Single-strand breaks
STS	Short-term starvation
TKIs	Tyrosine-kinase inhibitors
VEGF	Vascular endothelial growth factor

Contents

Introduction

In recent years, increasing evidences showed that several types of intermittent, chronic, or periodic dietary approaches, including short-term starvation (STS), long-term starvation (LTS or fasting), caloric restriction (CR), may exert a protective role against aging and other age-related pathologies as well as cancer in humans and numerous animal models (Lee and Longo 2016; Brandhorst and Longo 2016; Longo et al. 2015; Trepanowski et al. 2011). Interestingly, these dietary restriction (DR) regimens showed significant anticancer effects mostly in preclinical models, suggesting the possibility of using these methods to increase lifespan and improve therapy response in cancer patients. However, prolonged fasting periods could impair the patient health conditions already unfavorable due to physiological weight loss (Cleary and Grossmann 2011; Lluch et al. 2014). For this reason, STS (or intermittent fasting), consisting of the lack of food intake for a short time, appears to be the most suitable approach for cancer patients, although there are conflicting

opinions about it. STS aims to slow down growth of tumor, by restricting temporarily its exposure to different nutrients, including glucose, and generating protective effects against cancer (Robertson and Mitchell 2013; Anton and Leeuwenburgh 2013). Conversely, LTS consists of a prolonged food deprivation, resulting in adaptive cellular responses able to decrease inflammatory processes and oxidative stress, enhance energy metabolism, and strengthen cell protection (Longo and Mattson 2014). For example, a serum starvation able to bring down basal cellular activity was applied to several in vitro models, in order to study molecular mechanisms underlying apoptosis, cellular stress response, and autophagy (Pirkmajer and Chibalin 2011). Finally, CR is defined as the reduction in calorie intake aimed to inhibit tumorigenesis and prevent other diseases, including diabetes and cardiovascular pathologies, by inducing an improved insulin sensitivity and reducing the oxidative damage and metabolic rate (Lv et al. 2014; Lefevre et al. 2009).

This chapter aims to provide an overview of the most recent studies present in literature concerning the molecular mechanisms by which dietary restriction may contribute to prevent cancer onset, slow down its progression, and positively affect the response to anticancer therapies, also suggesting a close correlation between diet and reduction of treatment-induced side effects.

Molecular Pathways Involved in Dietary Restriction and Cancer-Related Events

Molecular Changes Induced by Dietary Restriction

Nowadays, the link between cancer and metabolism is becoming increasingly evident (Longo and Mattson 2014; Brandhorst et al. 2017). It is clear that beneficial effects mediated by fasting, in particular by CR, do not involve a single gene, a pathway or a unique molecular mechanism. The benefits are due to the negative regulation of nutrient-signaling pathways, including insulin-like growth factor 1 (IGF-1) pathway and its effector extracellular signal-regulated kinase (ERK), mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3K), which are known to modulate important proliferation pathways (Cangemi et al. 2016). Furthermore, it is well known that genomic instability is a distinctive feature of cancer, and CR tumor response seems to play a key role for the maintenance of genomic integrity (Robertson and Mitchell 2013; Duan et al. 2017). Due to these evidences, new metabolic approaches are being sought today for anticancer treatment. CR, also used in combination with the conventional chemotherapies, has allowed to obtain good results in animal models (Klement and Fink 2016).

CR-Induced Changes in Tumor Microenvironment

The most recent data in literature showed a correlation between aging and neoplastic diseases. It has been observed that aging promotes neoplastic cell growth and proliferation through surrounding microenvironment alterations. This process,

named "adaptive oncogenesis," is determined by tissue decline caused by age and oncogenic cell alterations (Cadoni et al. 2017). Changes in age-associated tissue microenvironment seem to play an important role in cancer and cancer-related diseases. Although the mechanisms responsible for delays in aging and carcinogenesis have not been fully identified, CR is today the only known nongenetic approach able to extend organism life. Nutrient-sensing pathways play a pivotal role in cellular response to CR probably because these regulatory processes are responsible for maintaining a microenvironment that promotes aging and carcinogenicity (Cadoni et al. 2017).

The deacetylase SIRT1 is a protein implicated in regulation effects downstream of CR, in both human and murine models (Cohen et al. 2004). The SIRT1 levels are low in senescent cells probably due to the formation of the C/EBPβ complex and HDAC1, which bind and inhibit SIRT1 promoter. Several studies showed that long-term CR is able to block the formation of the C/EBPβ and HDAC1 inhibitory complex, restoring the functionality of SIRT1 promoter in murine liver cells (Jin et al. 2011).

The SIRT1-activated pathway is also involved in the regulation of forkhead box subgroup O (FOXO) protein, which is deacetylated by SIRT1 in response to oxidative stress. The FOXO1 levels seem to be increased in rat liver cells during long-term CR. This suggests that both SIRT1 and FOXO1 have a modulating role in long-term CR and are responsible for creating a microenvironment that delays aging and prevent cancer (Yamaza et al. 2010).

DR and Inflammatory Response

Several studies showed that DR also plays a role in modulating inflammatory response. Liver cells of diethylnitrosamine (DEN)-induced HCC mice models submitted to DR showed a reduction in levels of NF-kB, a mediator of inflammation associated with cell proliferation and cancer (Duan et al. 2017). A decrease in levels of cytokines and inflammatory chemokines was observed in murine liver, kidney, and spleen tissues (Chiba and Ezaki 2010). Also, mice under 4 weeks DR condition display a reduction of proinflammatory gene expression and an increase in anti-inflammatory gene expression (Robertson and Mitchell 2013; Fig. 1).

DR and Chemotherapy Protection

Proliferation pathways regulated by Ras and AKT are almost always constitutively activated in cancer cells. Cells dramatically reduce the cell division number and become more resistant to stress in response to poor nutrition conditions, such as fasting or DR. This occurs because DR inactivates nutrient-sensing signaling pathways (Brandhorst et al. 2017). The link between cell proliferation, which depends on the nutrient-sensing pathways, and stress resistance is the basis of the protective effect that DR exerts on normal cells compared to tumor cells. This resistance is called differential stress resistance (DSR). In fact, tumor cells are unable to protect

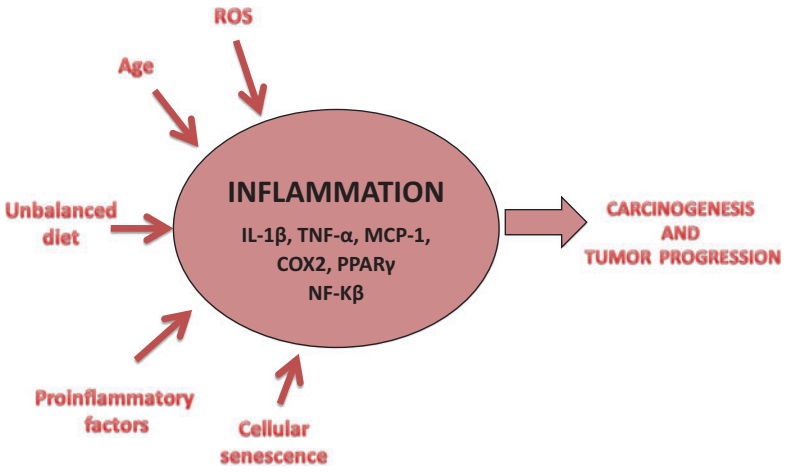

Fig. 1 **Association between inflammation and cancer**. Unbalance diet, age, cellular senescence, and accumulation of proinflammatory factors and ROS cause cell inflammation

themselves from stress, because oncogenes negatively regulate resistance genes (Brandhorst et al. 2017; Raffaghello et al. 2008). Moreover, several mutations accumulated in cancer cells make them less able to adapt to extreme environmental conditions created by fasting (Longo and Fontana 2010). Several studies showed that DR-induced DSR may be used to protect healthy cells from the toxic effects of chemotherapy (Brandhorst et al. 2017).

The fasting-induced protection has been shown also in in vivo experiments. CR protects mice from high dose etopoxide toxicity 4, nausea and vomiting induced by doxorubicin, and irinotecan-induced weight loss (Raffaghello et al. 2008; Tinkum et al. 2015). It has also been found that IGF-1 gene deletion protects against chemotherapeutic toxicity of doxorubicin and cyclophosphamide (Brandhorst et al. 2017). Probably, the protective effect induced by DR and starvation is due to a change in microenvironment of the intestinal cryptic stem cells. Indeed, fasting before chemotherapy preserves the correct architecture and functioning of intestinal cells by maintaining the expression of genes such as Lgr5, Bm1, and HopX (Tinkum et al. 2015).

In other studies, it was observed that DR makes cancer cells susceptible to cisplatin-based chemotherapy effects through activation of the ATM/ChK2/p53 signaling pathway, which causes temporary loss of coordination between cell proliferation and growth stimulated by nutrients (Shi et al. 2012).

Correlations Between DR and IGF-1, Insulin, and Cancer

One of the nutrition-related pathways involved in carcinogenesis is the IGF-1 signaling, which affects both sensitivity to oxidative stress and DR. Insulin and IGF-1 play a pivotal role in controlling metabolism and growth in response to

nutritional signals and nutritional state of cells (Shi et al. 2012). IGF-1 pathway regulates cell proliferation and differentiation, showing a tumorigenic effect through apoptosis inhibition (Ramsey et al. 2002; Prisco et al. 1999). Epidemiological studies highlighted the role that IGF-1 pathway plays in cancer pathology. Indeed, high serum IGF-1 concentration is associated with an increased risk of prostate, breast, and colon cancers (Renehan et al. 2004).

A study performed on murine xenograft models showed that deregulation of IGF-1 and PI3K/AKT pathways results in DR resistance. IGF-1 recruits PI3K on cell membrane via binding to tyrosine kinase receptor, resulting in AKT activation. AKT, in turn, phosphorylates and activates downstream effectors that induce cell proliferation (Kalaany and Sabatini 2009).

FOXO1 protein is an effector downstream of IGF-1/AKT pathway, negatively regulated by AKT. This protein is able to modulate the expression of genes involved in oxidative metabolism, stress resistance, and longevity (Cangemi et al. 2016). It has been observed that DR-sensitive cells show a decrease in AKT cytoplasmic levels. This results in FOXO1 nuclear relocation and induction of the proapoptotic and antiproliferative gene transcription. In addition, in vivo studies on xenograft models demonstrated that DR-induced apoptosis increases in tumor cells that overexpress FOXO1. These results are consistent with the antitumorigenic effect of FOXO1 in DR conditions (Kalaany and Sabatini 2009).

A significant role is played by downstream effectors of the PI3K pathway, such as mTOR, AMPK, and SIRT1, which are probably related to cellular sensitivity to DR. Moreover, it has been observed that mutations constitutively activating PI3K protein are important for tumor sensitivity to DR. In fact, an increased sensitivity to DR is observed when PI3K levels decrease. This suggests that molecular analysis of PI3K mutational state could represent an interesting tool to identify DR resistance markers (Lee et al. 2012b; Fig. 2).

Several epidemiological studies showed that there is a strong correlation between increased adiposity and tumor risk (Lee et al. 2012b; Fanale et al. 2017; Wang et al. 2012; Toren et al. 2013; La Paglia et al. 2017). Adiposity is associated with an increase in insulin serum levels. Insulin, an anabolic hormone produced by pancreatic β-cells, exhibits mitogenic effects on many cell types, especially on preneoplastic cells. Furthermore, insulin increases IGF-1 activity, reducing synthesis and secretion of IGF-binding protein 1 (IGFBP-1) (Esposito et al. 2003). Hyperinsulinemia increases concentration of circulating sex hormones, in particular stimulating the production of androgens involved in the growth of different tumors. Several studies showed that DR counteracts metabolic anomalies associated with excessive adiposity, by reducing insulin levels, sex hormones, IGF-1, inflammatory cytokines, prostaglandins, and other various markers of oxidative stress and DNA damage (Esposito et al. 2003, Heilbronn et al. 2006; Fig. 3). According to the data of epidemiological studies carried out on dietary style of Western countries, DR associated with low protein intake has been shown to decrease serum IGF-1 levels in humans (Fontana et al. 2008; Giovannucci et al. 2003).

DR in combination with deprivation of essential amino acids triggers protective events for cells, by inducing a decrease in mTORC1 cellular levels and a concomitant increase in amino acid deprivation sensor (GCN2) (Brandhorst et al. 2017).

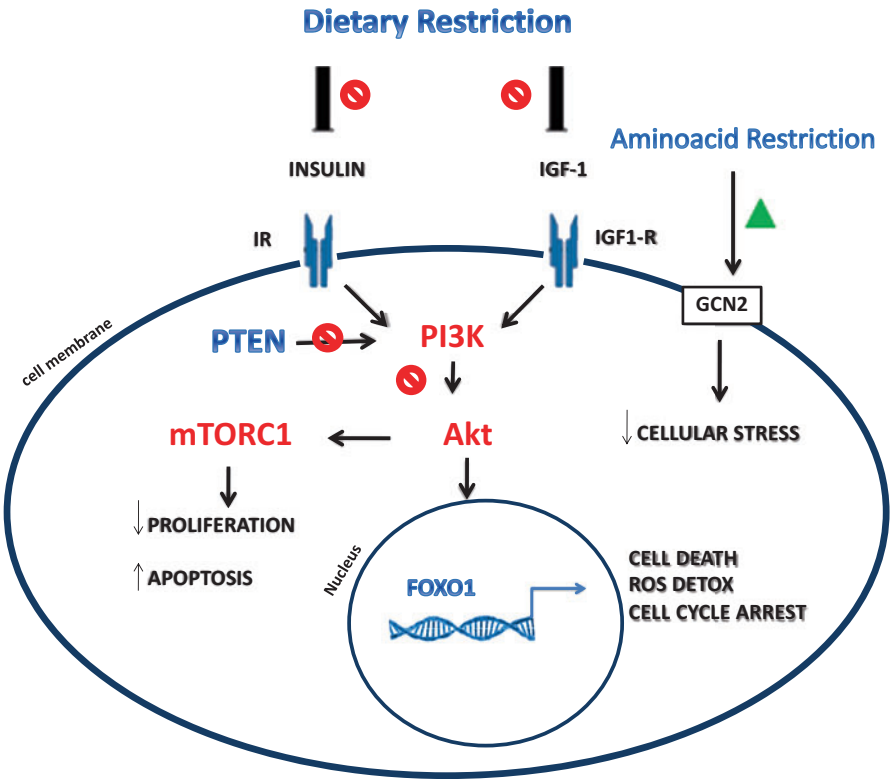

Fig. 2 Molecular pathways modulated by dietary restriction. Dietary restriction decreases the circulating levels of insulin and IGF-1, resulting in inhibition of PI3K/AKT pathway, and leading to increased apoptosis and decreased proliferation

Although there is still no data regarding the effect of fasting in preventing cancer in humans, the most likely hypothesis is that the effect of DR on IGF-1 levels could generate a protective environment for healthy cells and an adverse environment for tumor cell growth (Longo and Mattson 2014). Another protein restriction marker is FGF21, the fibroblast growth factor, regulated by PPARα whose plasma levels increase during DR associated with protein restriction. In summary, DR has effects on FGF21, IGF-1, and mTOR activity, which are probably linked to carcinogenesis (Klement and Fink 2016).

DR and Oxidative Stress Response

Many studies indicated that the increase of antioxidant factors in tumor cells is mediated by threonine tyrosine kinase Rim15 and transcription factors Msn2/4 and Gis1, which regulate several genes, including mitochondrial SOD2, implicated in

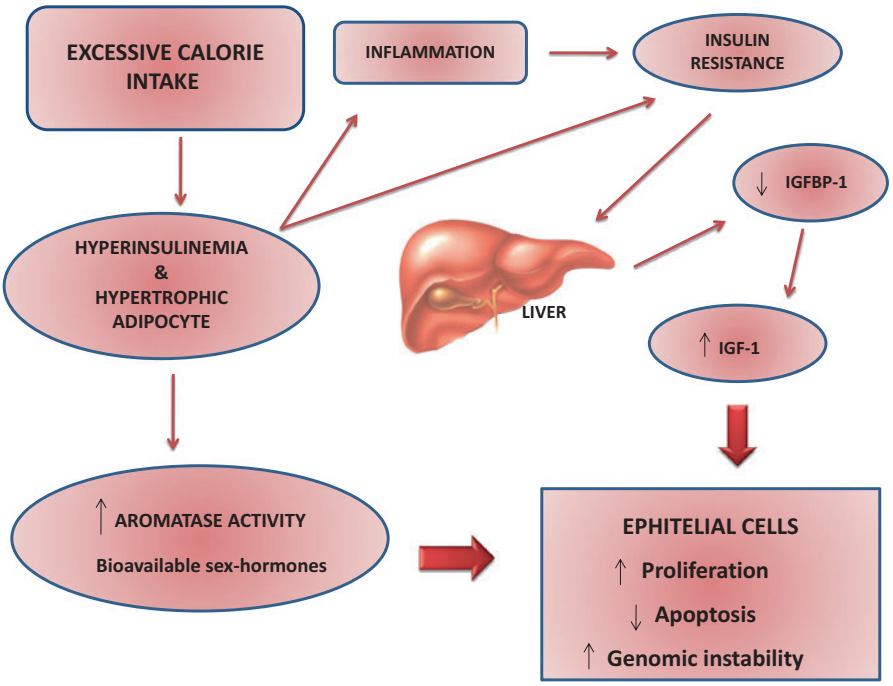

Fig. 3 Correlation between calorie intake and adiposity. Excessive calorie intake causes hyperinsulinemia, hypertrophy of adipose tissue, and increased inflammation

oxidative stress resistance (Madia et al. 2009; Hlavata et al. 2003). Additionally, Tor/Sch9 and Ras/AC/PKA pathways regulate the expression of several DNA repair genes, including REV1 gene (Madia et al. 2009).

According to this evidence, Mn-superoxide dismutase (MnSOD) heterozygous knockout mice showed an increased DNA oxidative damage and tumor incidence. This suggests a complex interaction between oxidative stress and cancer (Van Remmen et al. 2003).

Another mechanism induced by DR is the autophagy response to oxidative stress. Autophagy is a process by which cells under DR conditions convey nutrients to essential metabolic processes. DR-induced autophagy is activated by poly (ADP-ribose) polymerase 1 (PARP-1), a nuclear enzyme induced by DNA damage. ROS (reactive oxygen species) production under DR conditions causes DNA damage, which determines PARP-1 activation and fasting-induced autophagy (Cangemi et al. 2016). ROS induce different types of DNA damage, including single-strand breaks (SSBs), double-strand breaks (DSBs), and ionized DNA nucleotides. The repair of latter damage requires the intervention of the base excision repair (BER) system, in particular the OGG1 (8-oxoguanine DNA glycosylase) enzyme 1. Both in vitro and in vivo experiments showed that BER activity is influenced by the availability of nutrients. Indeed, autophagy has no effects on OGG1 expression in the absence of fasting (Siggens et al. 2012).

DEN-induced mice HCC cells exhibit high levels of Caspasi 3, PARP, and Citocromo C, which are proteins involved in mitochondria-mediated autophagy, suggesting that DR suppresses proliferation and promotes apoptosis (Lu et al. 2008; Duan et al. 2017).

According to previous studies (Xie et al. 2007; Standard et al. 2014), gene expression analysis of DEN-induced mice HCC cells revealed that DR restores functioning of many MAPK genes. MAPK pathway regulated by RAS promotes tumor growth and is one of the most important molecular targets for treatment of several types of cancers (Duan et al. 2017).

The Implication of Dietary Restriction in Cancer

The different molecular signature that distinguishes a normal cell from a tumor cell is the main reason that could explain the different susceptibility to growth stimuli. In fact, tumor cells undergo a series of genetic and epigenetic modifications that make their growth independent of the presence of growth factors (Hanahan and Weinberg 2011; Fig. 4). The accumulation of these genetic alterations constitutively activates key components of intracellular pathways. Among these, the most common deregulated signal pathways are Ras/Raf/MAPK and PTEN/PI3K/AKT, responsible for an uncontrolled cell proliferation (Massihnia et al. 2016). The deprivation of nutrients both in vitro and in vivo results in a decrease of growth factor levels in

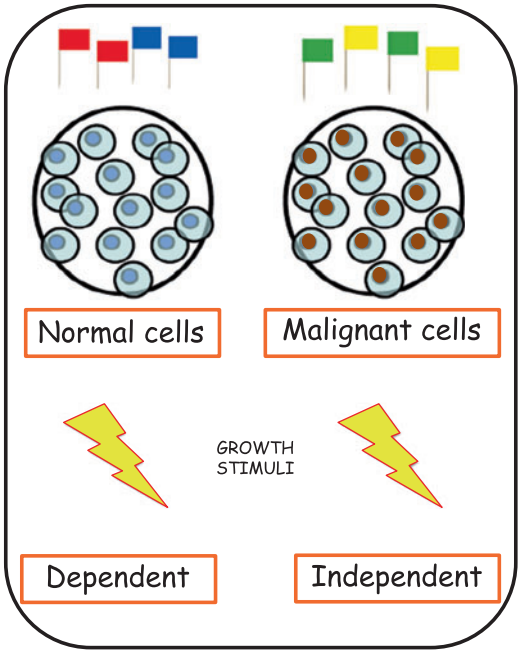

Fig. 4 Growth stimuli of normal and malignant cells. The different molecular profile between normal and malignant cells is responsible for the differences in behavior toward growth stimuli

normal cells, forcing thus the cell to enter a proliferative quiescent status (Flemstrom et al. 2010; Pirkmajer and Chibalin 2011). Unlike normal cells, tumor cells overcome this block by reprogramming their metabolic state and thus maintaining high proliferative abilities (Hanahan and Weinberg 2011). The discovery that different types of dietary restrictions can protect normal cells from the most common side effects of chemotherapy has recently raised interest in its possible clinical application. Moreover, STS seems to protect not only healthy cells but also increase the sensitivity of various types of cancer to the therapy (Lee and Longo 2011). Indeed, fasting in combination with chemotherapy determines increased cytotoxic effects in malignant cells from different types of cancers (Russo and Rizzo 2008). The validity of STS has been evaluated in immunosuppressed nude mice xenograft models in which human neuroblastoma cells were subcutaneously injected. Surprisingly, after 34 days of fasting combined with cyclophosphamide treatment the tumor mass was reduced (Lee et al. 2012a). The usefulness of STS has also been demonstrated for its attenuating properties on chemotherapy side effects. Indeed, its cardioprotective properties have been recently demonstrated during doxorubicin-based treatments (Dirks-Naylor et al. 2014). In addition, a recent work on murine models revealed that following a prolonged fasting of 48–60 h prior to the administration of a high dose of etoposide, the side effects, generally resulting from the treatment, were attenuated (Raffaghello et al. 2008). The synergy between refeeding and DNA damage caused by pharmacological treatment may favor the growth of new foci in various organs including liver, colon, and rectum (Laconi et al. 1995; Premoselli et al. 1998). Interestingly, in a tumor mass, malignant cells are strictly connected with the so-called "cancer-associated adipocytes" (CAAs) and interact with them (Calle and Kaaks 2004). In particular, CAAs show the reduction of peculiar markers including HSL, APN, and resistin, and increased proinflammatory cytokine expression such as IL-6 and IL-1β and TNF-α (Berstein et al. 2007; Ribeiro et al. 2012; Dirat et al. 2011). This altered expression, associated with the production of adipokines, results in a tumor microenvironment variation that favors uncontrolled growth. Therefore, fasting, having a massive effect on the size of adipocytes, can consequently decrease the secretion of tumor-favorable factors (Hermsdorff et al. 2009). Recently, CR efficacy has also been demonstrated in relation to radiotherapy, leading to an increase in the sensitivity to radiation-induced cytotoxicity (Champ et al. 2013). As alternative to standard chemo−/radiotherapy, another type of metabolic therapy has been proposed (ketogenic diet) whose beneficial effects have been demonstrated in the multiform glioblastoma and brain cancer for its antiangiogenic, anti-inflammatory, and antiapoptotic abilities (Seyfried et al. 2015). Below we will discuss deeper the association of chemotherapy and dietary restriction in some of the most spread cancers worldwide looking at the benefits deriving from their combination (Fig. 5).

Breast Cancer

Breast cancer is one of the main causes of cancer deaths in the female population (Fanale et al. 2013). Various clinical studies have shown the efficacy of fasting in the

Fig. 5 Association between dietary restriction and chemotherapy. The figure shows the relevant benefits arising from the association between different types of dietary restriction and drug administration

favorable outcome of the chemotherapy treatment to which some patients affected by breast cancer have undergone. In particular, it seems that a short period of fasting pre- and posttreatment will have a better outcome in terms of patient's tolerability by reducing the side effects. Indeed, the case report of three different patients treated with different therapies and subjected to different times of fasting is below described. A first woman of 51 years with a breast cancer at stage 2A did not show any side effect once subjected to fasting 140 h before and 40 h after treatment with docetaxel and cyclophosphamide. The validity of the association was confirmed in a second 53-year-old patient, also suffering of a tumor in stage 2A and HER2+. In particular, chemotherapy cycles associated with fasting 64 h before and 24 h after were not accompanied by high toxicity effects or in any case with negligible and/or reversible transient effects. The third case saw a 78-year-old patient with a HER2+ tumor, after mastectomy and subjected to variable fasting periods in the course of carboplatin-based, docetaxel and trastuzumab-based chemotherapy cycles. Significant levels of pharmacological toxicity have not been reported (Safdie et al. 2009; Table 1).

Ovarian Cancer

Among gynecological tumors, ovarian cancer is one of the most common and the fifth cause of death in the female population (Reid et al. 2017). Al-Wahab et al. (2014) published a study showing the effects of energy balance in mouse models

Table 1 Case reports of different tumors. The table summarizes the cases described in the text and related fasting schedules adopted in pre- and posttreatment

Breast cancer	Case I (51yo)	140 h before 40 h after treatment
	Case II (53yo)	64 h before 24 h after Treatment
	Case III (78yo)	Not shown
Ovarian cancer	Case I (44yo)	62 h before 24 h after treatment
Lung cancer	Case I (61yo)	48 h before 24 h after treatment
Prostate cancer	Case I (74yo)	60 h before 24 h after treatment

subjected to high energy diet or CR conditions. Mice group under high-energy diet showed the most extensive tumor formation accompanied by the highest tumor score at multiple sites. Moreover, they showed increased levels of insulin, leptin, IGF-1, VEGF, and proinflammatory factors (IL-6). Instead, the mice group under CR showed a lower tumor burden as well as a great reduction in insulin, IGF-1, leptin, MCP-1, VEGF, and IL-6 levels (Al-Wahab et al. 2014). Also, clinical trials demonstrated the effectiveness of the association chemotherapy/DR. The case of a 44-year-old woman suffering from ovarian cancer has been emblematic, because she has benefited from the antineoplastic treatment in combination with STS carried out 62 h before and extended 24 h after drug treatment (Safdie et al. 2009; Table 1).

Lung Cancer

Lung cancer is one of the major causes of cancer-related morbidity and death in men and women population worldwide. Depending on EGFR mutational status, therapy may vary in favor of tyrosine-kinase inhibitors (TKIs). Among them, erlotinib is one of the most commonly used TKIs in I and II line of treatment (Passiglia et al. 2017). Currently, the recommended dose is 150 mg under complete fasting conditions or 2 h after the meal consumption. Two modalities of administration are resulted able to determine a different drug absorption and consequent increase in therapeutic efficacy. In particular, drug seems to have greater effect when the administration takes place 2 h after the meal (Katsuya et al. 2015). An interesting clinical case is that of a 61-year-old NSCLC patient who has seen mitigating the side effects of drug therapy after STS 48 h before and 24 h after therapy (Safdie et al. 2009; Table 1).

Prostate Cancer

Prostate cancer is considered as the second cause of cancer-related death in male population (Vanacore et al. 2017). Interesting is the case of a 74-year-old man

diagnosed with stage 2 prostate adenocarcinoma. The patient has undergone several cycles of chemotherapy during which he faced many side effects such as fatigue, weakness, short-term memory impairment, and peripheral neuropathy. Moreover, PSA levels raised with an unstoppable trend. After many failed attempts, patients were enrolled in a rigorous fasting schedule consisting of restrictions 60 h prior to and 24 h post drug administration. After treatment, PSA levels dropped dramatically and a marked reduction of side effects was reported (Safdie et al. 2009; Table 1).

Conclusion

Recently, accumulating evidence showed that starvation condition seems to play a pivotal role in preventing cancer development and progression as well as improving the response to different therapeutic treatments. The above presented studies and considerations seem to confirm the protective role exerted by fasting against cancer. However, a potential limitation is represented by time required for each cancer patient to obtain an optimal fasting condition, since a severe nutrient deprivation for several months may be needed, as showed by in vivo preclinical analyses. Furthermore, not all patients are fit to undergo such dietary regimens, because many of them are subject to weight loss due to the chemotherapy toxicity and tumor itself. For this reason, since CR and ketogenic diet (KD) have been shown to be approaches particularly effective unlike intermittent fasting whose role is still controversial, further clinical studies are yet necessary in order to assess the safety and efficacy of these methods.

Policies and Protocols

Protocol for Maintaining Cancer Cells Under Short-Term Starvation Conditions

In this chapter, we have discussed the most significant studies present in literature concerning the molecular mechanisms by which dietary restriction may contribute to prevent cancer development, slow down its progression, and positively influence therapy response. Since most of the current experimental evidences concerning the correlation between dietary restriction and cancer arise from studies mainly performed on in vitro preclinical models, here we discuss a rapid and convenient method for maintaining the healthy and malignant breast cells under short-term starvation conditions restricting the supply of glucose. Glucose metabolism represents a primary source of energy able to support cell proliferation and regulate cell death-related signaling pathways. The impaired balance between excessively high glucose consumption and its poor supply determines glucose deprivation in the tumor microenvironment, activating a positive feedback mechanism that involves

ROS production by NADPH oxidase and mitochondria, inhibition of tyrosine phosphatases by oxidation, and amplification of tyrosine kinase signaling in cells dependent on glucose for their survival.

Both cell lines are grown at 37 °C, 5% CO2, and 80% confluence, in a culture medium DMEM (Dulbecco's Modified Eagle Medium) containing high glucose concentration (4.5 g/l D-glucose, 110 mM pyruvate) and enriched with fetal bovine serum (10% FBS), nonessential amino acids (NEAA-1%), and streptomycin-penicillin (1% Strepto/Pen). For short-term starvation experiments aimed at establishing a glucose deprivation, cells are washed twice with PBS (phosphate-buffered saline) and then incubated in glucose-free DMEM without pyruvate and supplemented with 10% FBS, 1% NEAA, 1% Strepto/Pen.

Dictionary of Terms

- **Caloric restriction** – Reduction of calorie intake that implicates feeding once daily or thrice weekly. It can be distinguished in two forms: intermittent caloric restriction and chronic caloric restriction.
- **Dietary restriction** – Condition of short- or long-term fasting which can involve the lack of food consumption for short or prolonged periods. It includes the caloric restriction, short- and long-term starvation, and ketogenic diet.
- **Hyperinsulinemia** – The increase in circulating insulin levels that lead to a greater IGF-1 activity, by reducing synthesis and secretion of IGF binding protein 1, and elevated concentration of circulating sex hormones.
- **Inflammatory response** – Set of actions exerted by the immune system to fight an inflammation through release of several factors that mediate this response, including chemokines and cytokines.
- **Therapy response** – Assessment of the extent of sensitivity or resistance of a tumor to a specific anticancer treatment.

Summary Points

- This chapter focuses on molecular mechanisms by which dietary restriction may contribute to prevent cancer onset and improve therapy response.
- Dietary restriction involves food deprivation for short or prolonged periods.
- Dietary restriction includes short-term starvation, long-term starvation, caloric restriction, and ketogenic diet.
- Numerous experimental evidences showed that fasting may exert a protective role against aging and other age-related pathologies as well as cancer.
- Preclinical models suggested the potential use of fasting to induce anticancer effects and improve patient life's quality.
- However, prolonged fasting periods could impair the patient health conditions already unfavorable due to physiological weight loss induced by tumor itself.

- Fasting-mediated benefits seem to be mediated by the reduction of inflammatory response and down-regulation of nutrient-related signaling pathways able to modulate cell proliferation and apoptosis.
- Good results were obtained in animal models by associating caloric restriction with conventional chemotherapies.
- Deregulation of IGF-1 and PI3K/AKT pathways causes dietary restriction resistance.
- This chapter describes some case reports for different types of cancer.

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