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# Starvation, detoxification, and multidrug resistance in cancer therapy

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ABSTRACT

The selection of chemotherapy drugs is based on the cytotoxicity to specific tumor cell types and the relatively low toxicity to normal cells and tissues. However, the toxicity to normal cells poses a major clinical challenge, particularly when malignant cells have acquired resistance to chemotherapy. This drug resistance of cancer cells results from multiple factors including individual variation, genetic heterogeneity within a tumor, and cellular evolution. Much progress in the understanding of tumor cell resistance has been made in the past 35 years, owing to milestone discoveries such as the identification and characterization of ABC transporters. Nonetheless, the complexity of the genetic and epigenetic rewiring of cancer cells makes drug resistance an equally complex phenomenon that is difficult to overcome. In this review, we discuss how the remarkable changes in the levels of glucose, IGF-I, IGFBP-1 and in other proteins caused by fasting have the potential to improve the efficacy of chemotherapy against tumors by protecting normal cells and tissues and possibly by diminishing multidrug resistance in malignant cells.



## 1. Dietary restriction, nutrient signaling, and stress resistance

Dietary restriction triggers highly conserved survival mechanisms that enhance the protection of organisms ranging from bacteria, yeast, worms, flies, and mice, to non-human primates against various types of stress and/or disease. This counterintuitive effect is mediated in part by the reduction of conserved nutrient-signaling pathways that include several mitogenic components, especially the IGF-I receptor and its downstream effectors (Fontana et al., 2010; Guarente and Kenyon, 2000; Kenyon, 2010; Longo, 1999; Longo and Finch, 2003). In this section, studies that establish the link between starvation conditions and the conserved nutrient-signaling pathways in eukaryotes with enhanced cellular protection in a variety of organisms will be discussed (Fig. 1).

In *Escherichia coli*, carbon-starvation promotes the synthesis of stress resistance proteins, including heat-shock proteins, as determined by 2-D gel electrophoresis analysis (Matin, 1991). In fact, glucose or nitrogen starvation enhances the resistance of *E. coli* to heat (57 °C) or $H_2O_2$ (15 mM) (Jenkins et al., 1988). Furthermore, glucose restriction not only increases the expression of alternate carbon/energy and nitrogen sources, but it also increases components of the stress response regulons (Hua et al., 2004; Ihssen and Egli, 2004; Wick et al., 2001). Similar effects were reported in *Salmonella typhimurium* (Seymour et al., 1996). In many bacterial species, starvation induces the stationary-phase specific sigma factor RpoS expression, which in turn controls a large set of stress defense genes, in particular oxidative stress (McDougald et al., 2002; O'Neal et al., 1994).

In the yeast *Saccharomyces cerevisiae*, incubation in reduced glucose (2.0–0.5 g/L) promotes markedly increased resistance to oxidative stress, and starvation achieved by incubation in water promotes a major increase in life span as well as protection from oxidative insults and heat shock (Longo et al., 1997; Wei et al., 2008). Also, deficiency in the nutrient-sensing Ras/cAMP/PKA and/or the Tor/S6K pathways (Thevelein and de Winde, 1999; Wei et al., 2008) more than doubles lifespan and protects against a variety of stresses (Fabrizio et al., 2001, 2003; Longo and Finch, 2003; Wei et al., 2009). The stress responsive transcription factors Msn2/Msn4 and Gis1, and the genes regulated by them, mediate much of this protection, as yeast that lack these factors no longer exhibit enhanced stress resistance (Longo et al., 1997; Wei et al., 2008). Down-regulation of these pro-growth pathways and up-regulation of the Msn2/4 and Gis1 transcription factors also represents a major component responsible for the effects of dietary restriction (DR) and starvation on stress resistance and longevity (Wei et al., 2008). Among the genes regulated by these transcription

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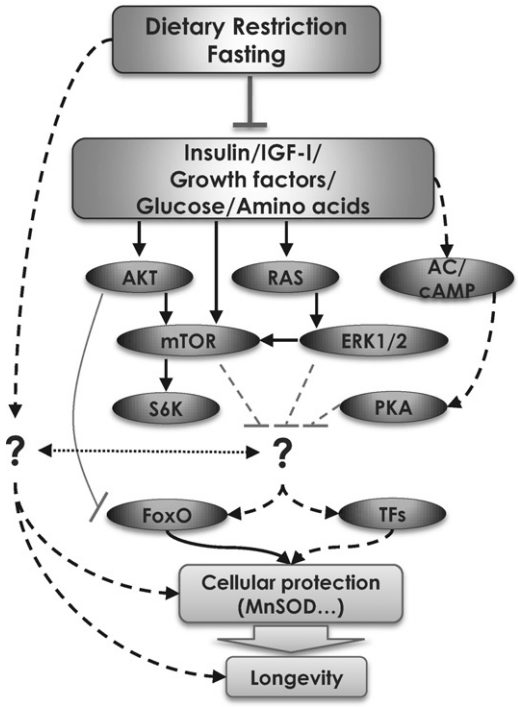


**Fig. 1.** The conserved role of IGF-I in lifespan regulation and stress resistance. The insulin/IGF-I signaling pathway plays a major role in regulating lifespan and cellular stress resistance. Deficiencies in these pathways have been shown to convey protection to the cell/organism against multiple toxins.

factors that contribute to stress resistance are heat shock proteins, MnSOD, and metabolic enzymes that generate glycerol (Wei et al., 2009).

In worms, starvation conditions also enhance protection against oxidative stress. When grown under DR, achieved by feeding the animals with diluted bacteria, worms increase their resistance to paraquat (Greer et al., 2007). Every 2-day fasting also increases resistance to oxidative and heat stress, and extends the lifespan of worms by up to 56% via modulation of the RHEB-1 and TOR signaling pathway, both of which are linked to the FOXO transcription factor homolog DAF-16 (Honjoh et al., 2009; Weinkove et al., 2006). Conversely, excessive glucose shortens the lifespan of worms, in part, by decreasing DAF-16 activity (Lee et al., 2009). Further, AGE-1 (PI3K homolog) and DAF-2 (insulin/IGF-I receptor homolog) mutants live up to twice as long by decreasing AKT-1/2 signaling, and by activating the transcription factor DAF-16/FoxO, and promoting resistance to oxidative and ER stress (Henis-Korenblit et al., 2010; Hsu et al., 2003; Johnson, 1990; Paradis et al., 1999). AGE-1 deficiency confers increased survival when challenged with 3 mM $H_2O_2$ (9-fold), paraquat (5-fold), and 4-hydroxynonenal (4-HNE; 4.4-fold) (Ayyadevara et al., 2008; Larsen, 1993; Morris et al., 1996). These longevity mutations are also associated with the induction of stress resistance transcription factors, mitochondrial superoxide dismutase (MnSOD), and HSPs in worms (McColl et al., 2010).

The fruit fly *Drosophila melanogaster* is generally fed a mixture of sucrose (carbon source) and yeast (protein source) in laboratory settings. Moderate DR (3% sucrose and 3% yeast vs the standard 10% sucrose and 10% yeast) but also amino acid restriction enhances the protection against oxidative damage following chronic hypoxia (Vigne and Frelin, 2006, 2007). Furthermore, a close to starvation condition, induced by feeding 1% sucrose and 1% yeast, but not moderate DR, protects flies from anoxia/reoxygenation injury (Vigne et al., 2009). Starvation-dependent protection against oxidative stress in flies is mediated by d4E-BP, which acts downstream of the PI3K/Akt/dFOXO3 pathway (Tettweiler et al., 2005), and also binds to eIF4E and represses translation, a mechanism consistent with the necessity of stressed cells to divert energy from growth to protection. Also, the insulin-like receptor (InR) and its downstream substrate *chico* regulate longevity and stress resistance. *Chico* was first identified as the insulin receptor substrate that regulates cell size and metabolism (Cicchetti et al., 2009), and loss of its activity increases lifespan and provides some resistance to paraquat (Clancy et al., 2001; Giannakou and Partridge, 2007). In flies, there are 7 genes encoding for insulin-like ligands (*Drosophila* insulin-like peptides; DILP 1–7). The reduction of DILPs increases lifespan and resistance against paraquat and heat (Ikonen et al., 2003).

Laboratory rodents also display increased stress resistance in response to DR. Mice under a 30–50% decreased dietary intake showed diminished levels of age-dependent lipid (Chipalkatti et al., 1983; De et al., 1983; Koizumi et al., 1987), protein (Dubey et al., 1996; Lass et al., 1998), and DNA oxidation (Chung et al., 1992; Kang et al., 1998). As discussed in more detail later, in mice, fasting for 48–60 h increases protection of 3 different strains of mice from etoposide, a drug known to promote oxidative stress, with remarkable improvement in survival compared to its normally fed counterparts (Raffaghello et al., 2008). In addition, 72-h of fasting protects the outbred CD-1 mice from lethal doses of doxorubicin, a drug also known to cause death by oxidative stress induced cardiotoxicity (Lee et al., 2010). Studies also show that fasting protects against ischemia reperfusion injury (IRI), a pathological condition initiated by a lack of blood flow (ischemia) and followed by restoration of blood flow (reperfusion) that causes further damage by inappropriate activation of cellular oxidases and subsequently by inflammatory proteins (Friedewald and Rabb, 2004), in rat brain (Go et al., 1988; Marie et al., 1990), mouse kidney and liver (Mitchell et al., 2010; van Ginhoven et al., 2009), and in human liver (van Ginhoven et al., 2009). Also, fasting following traumatic brain injury proved to be neuroprotective, resulting in reduced oxidative damage and improved cognitive function (Davis et al., 2008). Furthermore, it has been reported that intermittent fasting (IF), during which mice are fed every other day, can protect heart and brain cells against injury and improve functional outcome in animal models of myocardial infarction and stroke (Ahmet et al., 2005; Mattson and Wan, 2005; Wan et al., 2010). Deficiency in certain amino acids has also been shown to promote protection in mice. For instance, methionine restriction dramatically increases the hepatic resistance against acetaminophen-induced oxidative damage, and increases maximum lifespan and reduces age-dependent deterioration (Miller et al., 2005).

In mice, the GH/IGF-I axis and its downstream effectors, many being orthologs of the lower eukaryotes' proteins mentioned above, regulate stress resistance and lifespan (Bonkowski et al., 2009; Brown-Borg et al., 1996; Coschigano et al., 2000; Holzenberger et al., 2003; Migliaccio et al., 1999; Selman et al., 2009; Yan et al., 2007). Notably, it has recently been shown that the PI3K pathway, which represents a major arm of IGF-I signaling, is important in determining the sensitivity of tumors to DR, and mutations in the pathway may influence the response of cancers to DR. A recent study has shown that dietary-restriction-resistant tumors harbor a mutation that causes PI3K to be constitutively active. Replacing the mutant PI3K allele with a wild-type PI3K, or reintroducing PTEN expression in a PTEN-null cancer, was sufficient to reverse the resistance to DR (Kalaany and Sabatini, 2009) in agreement with the starvation-dependent differential stress resistance of normal and cancer or cancer-like cells to chemotherapy demonstrated in both yeast cells and mammalian cells and discussed in more details later in the review (Lee et al., 2010; Raffaghello et al., 2008).

Consistent with the effect of DR and starvation in reducing growth factor signaling, fibroblasts isolated from mice deficient in the GH/IGF-I axis are resistant to oxidative stress ($H_2O_2$, paraquat), UV, genotoxins (methylmethanesulfonate; MMS), heat, and

cadmium (Murakami, 2006; Salmon et al., 2005). Conversely, exposure of murine hepatocytes to GH or IGF-I or the overexpression of GH in transgenic mice reduces the levels of superoxide dismutases and catalase activity (Brown-Borg and Rakoczy, 2000; Brown-Borg et al., 2002). Similarly, IGF-I attenuates cellular stress response and the expression of stress response proteins HSP72 and heme-oxygenase in rats (Sharma et al., 2000). Modulation of the downstream elements of the IGF-I receptor (IGF-IR) alter stress resistance. For instance, MEK/ERK1/2 inhibition protects neurons against oxidative stress (Li et al., 2008), persistent PI3K-mTORC1 signaling decreases cellular stress resistance (Sun et al., 2011), and the inhibition of PI3K protected murine microglia against oxygen-glucose deprivation (OGD) stress (Chern et al., 2011) and also protected PC12 cells from serum-deprivation induced death (Guillon-Munos et al., 2005).

With special regards to cancer, GH/IGF-I axis alterations independent of nutrition and its dysfunction have been well documented. For instance, an unbalanced relationship between GH and IGF-I, as well as its binding protein IGFBP-3 has been suggested to play a major role in tumor biology (Luo et al., 2005; Mazzoccoli et al., 1999, 2010). However, the isolated reduction of circulating IGF-I levels alone, achieved by hepatic *igf1* gene deletion, has been shown to be insufficient in retarding prostate cancer progression leading to the suggestion that GH levels also need to be reduced (Anzo et al., 2008). However, IGF-I and the IGF-I receptor (IGF-IR) are well known to play a major role in the progression of a variety of tumors (Anzo et al., 2008; Braconi et al., 2008; Gong et al., 2009; Pollak et al., 2004).

## 2. Dietary restriction, nutrient signaling, and cellular detoxification

Drug metabolism can be largely divided into 3 phases: phase I (redox and hydrolysis), phase II (conjugation), and phase III (transport). Phase III transporters include the ABCB1 pumps which are discussed in more detail later. Diet and nutrient signaling pathways have profound effects on phase I enzymes, which consist primarily of cytochrome P450 (CYP) superfamily proteins (Yang et al., 1992) and phase II proteins including enzymes regulated by Keap1-Nrf2 (Kohle and Bock, 2007) such as UDP-glucuronosyltransferases (UGT) (Innocenti et al., 2002; Tukey and Strassburg, 2000), NAD(P)H:quinone oxidoreductase (NQO), superoxide dismutase (SOD) (Zhu et al., 2005) and glutathione-*S*-transferase (GST) (Belinsky and Jaiswal, 1993; Kong et al., 2001; Townsend and Tew, 2003). Notably, the Nrf2 pathway has been shown to mediate the anticarcinogenic effects of DR, but not its longevity effects, suggesting that it may function only to regulate specific systems activated by DR including those involved in cellular stress resistance (Choi et al., 2007). In fact, Nrf2 also mediates endothelial protection against oxidative stress induced by resveratrol, a natural polyphenol that promotes protection and longevity in model organisms (Zhai et al., 2005). As discussed above, longevity and stress resistance is heavily regulated by the insulin/IGF-I pathway, and certain downstream elements including ERK, JNK, p38 MAPK, and PI3K, which are known to regulate several detoxification enzymes (Xu et al., 2005). Fasting, DR, and the long-lived and stress resistant mutants discussed above appear to have potent detoxification effects although the mechanisms are poorly understood (Fig. 2). In worms, the extremely resistant spore-like dauer larvae and mutations in Daf-2, the worm ortholog of the insulin/IGF-I receptor up-regulate cytochrome P450, UGT, the small heat shock protein/α-crystallins, and GST (McElwee et al., 2004). Also, they exhibit increased ROS detoxification via the cytosolic antioxidant SOD enzyme (Vanfleteren, 1993; Vanfleteren and De Vreese, 1995)

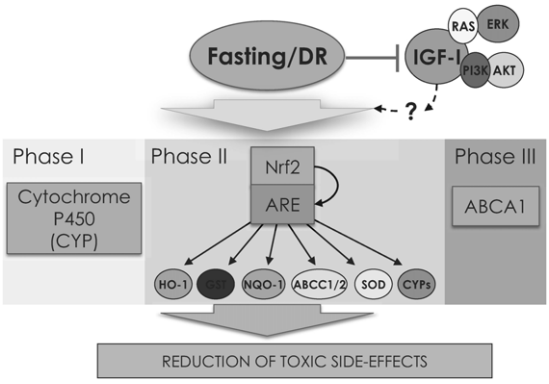


**Fig. 2.** Fasting and DR modulate drug metabolism. Fasting and DR have been shown to affect all 3 major phases of drug metabolism. Phase I involves redox and hydrolysis and is mediated by the cytochrome P450 family. Phase II are usually detoxification processes that involves conjugation steps. Genes under this category are regulated by the stress responsive master transcription factor Nrf2. Proteins classified as phase III are involved in transport, such as members of the ABC superfamily of transporters.

and the mitochondrial MnSOD (Honda and Honda, 1999; McElwee et al., 2003; Murphy et al., 2003).

DR, in worms, up-regulates the stress-responsive cytoprotective transcription factor SKN-1 (Nrf2 ortholog) which is required for its longevity (Bishop and Guarente, 2007). In flies, cytochrome P450 activity increases following 30 days of DR (Pletcher et al., 2005), whereas in mice, fasting for 48 h modulates hepatic expression of several ABC transporters and cytochrome P450s (Bauer et al., 2004). Similar to worms, DR induces Nrf2 activity in mice, which has been shown to be responsible for most of its anticarcinogenic effect (Martin-Montalvo et al., 2011).

IGF-I signaling has also been shown to be involved in the regulation of Nrf2. PI3K inhibitors block nuclear translocation of Nrf2 and induction of stress proteins (Kang et al., 2002; Nakaso et al., 2003; Shen et al., 2004). Also, a recent report suggests that Nrf2 and IGF-I are involved in cooperative effects (Wang et al., 2011). In human cancer cells, mutations in the coding region of *NRF2* cause constitutive induction of drug efflux pumps and cytoprotective enzymes (Shibata et al., 2008) such as NQO (Belinsky and Jaiswal, 1993). Further, IGF-I, and its downstream elements such as RAS/ERK (Nakamura et al., 2006) and PI3K/AKT (Abdul-Ghani et al., 2006; Han et al., 2007; Tazzari et al., 2007) promote MDR, in part, by modulating ABC transporter expression. Notably, DR increases Nrf2 in normal cells (Martin-Montalvo et al., 2011) and thus has the potential to be an important mediator of the differential effects of DR in the protection of normal and cancer cells (Lee and Longo, 2011; Raffaghello et al., 2008). Considering that fasting reduces IGF-I levels and promotes enhanced protection to chemotherapy in normal cells (Lee and Longo, 2011; Lee et al., 2010), it will be important to understand the link between different types of dietary restriction, growth signaling and detoxification enzymes.

## 3. Dietary restriction and MDR

Resistance to multiple categories of chemotherapy, also known as multidrug resistance (MDR), is a complex hurdle in cancer treatment. The mechanistic details behind multidrug resistance (MDR) are still under active investigation, but members of the ATP binding cassette (ABC) transporter superfamily, that often act as cellular efflux pumps for a wide range of chemotherapeutic compounds, have well-established roles (Calcagno et al., 2007; Gottesman et al., 2002). There are at least 48 MDR genes described, of which the ABCB1 gene, encoding an efflux transporter P-glycoprotein (PGP) or MDR1, is probably the most understood, closely followed by ABCC1 (MRP1), and ABCG2 (MXR and BCRP) (Gillet et al., 2004).

It appears that fasting for 24 h in 12–16 week old mice does not alter hepatic expression of many ABC family members, including *Abcb1*, but it induces the expression of *Abca1* (2.3-fold), involved in cholesterol transport, and *Abcg8* (1.8-fold), involved in sterol transport (van den Bosch et al., 2007). The ATP-binding cassette transporter A1 (ABCA1) has also been shown to promote MDR in addition to its more regular role as a regulator of hepatic cholesterol levels (Gillet et al., 2004; Iwasaki et al., 2010). Because malignant cells respond poorly or are indifferent to fasting, whereas normal cells rapidly adapt by regulating a large number of genes, the up-regulation of MDR proteins in the liver during fasting may contribute to reducing toxic side-effects. However, another study reports that ABCA1 expression can be repressed by a short fasting (20 h) in 6–8 week old mice (Rodgers and Puigserver, 2007). Thus additional studies on the effect of longer periods of fasting which are effective in promoting multi-stress resistance (48–72 h, see following section) on ABC transporter expression and host protection are necessary.

In an effort to overcome MDR, Gottesman and colleagues developed multidrug resistant bone marrow cells by transfecting them with vectors carrying the MDR1 cDNA allowing the use of unusually high doses of chemotherapy. High-dose chemotherapy, accompanied by bone-marrow transplant, is used to overcome MDR by overwhelming the PGP pump and increasing intracellular drug concentration (Makatsoris and Seiden, 1997; Patel and Rothenberg, 1994). The possibility that fasting may contribute to the differential chemotherapy protection by inducing specific MDR proteins in a variety of normal cells including bone marrow cells, but not cancer cells, remains to be tested (Lee et al., 2010; Raffaghello et al., 2008; Safdie et al., 2009).

Clinical approaches to overcome MDR in cancer cells include the use of chemotherapeutics that are not substrates of ABC transporters (e.g. cyclophosphamide, 5-fluorouracil, and Herceptin) and also combination therapy with non-toxic compounds that inhibit ABC transporters, also known as MDR inhibitors, MDR modulators, MDR reversal agents or chemosensitizers (McHugh and Callaghan, 2008; Ozben, 2006; Polgar and Bates, 2005). However, clinical data show that most inhibitors (first, second, and third generation MDR inhibitors) are not providing major improvements over the chemotherapy alone, suggesting that new combination therapies with wider effects may be required (Dong and Mumper, 2010; Hall et al., 2009; McHugh and Callaghan, 2008; Ozben, 2006). Because starvation conditions affect so many protective systems, it will be important to combine fasting with lower and non-toxic doses of these MDR inhibitors to test the hypothesis that this combination may reduce the toxic side-effects in normal tissues while reversing the resistance of cancer cells.

## 4. Starvation (STS) and differential stress resistance (DSR)

A concern arising from the fasting-based methods to protect normal cells is the possibility that malignant cells may also be protected. However, fundamental differences between normal and malignant cells are the basis for the starvation-dependent differential stress resistance (DSR) hypothesis, which proposes that specific starvation conditions will protect normal cells against chemotherapy without interfering with the killing of malignant cells (Lee et al., 2010; Raffaghello et al., 2008) (Fig. 3). In fact, we have recently shown that fasting alone can cause cytotoxicity of many different types of tumors, which further increases when combined with chemotherapy (Lee et al., 2012). Cancer cells have undergone a series of genetic and epigenetic alterations. Two of the major alterations in cell physiology that dictate malignant growth are: (1) self-sufficiency in growth signal, and (2) insensitivity to anti-growth signals (Hanahan and Weinberg, 2000). In fact, the great majority of normal cells are unable to grow in the absence of growth

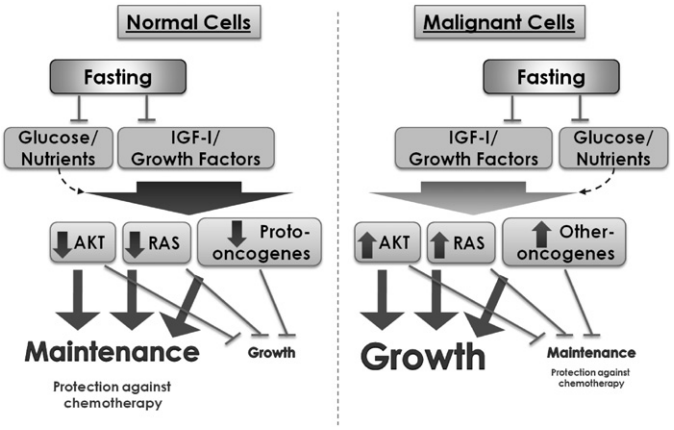


**Fig. 3.** A model for DSR in response to fasting. In a variety of normal cells, downstream elements of the IGF-I and other growth factors pathways, including the Akt, Ras and other proto-oncogenes, can be down-regulated in response to the reduction in growth factors caused by starvation. This down-regulation can block/reduce growth and promotes protection. By contrast, oncogenic mutations render tumor cells less responsive to fasting due to their constitutive pro-growth mode and relative independence from anti-growth signals. Constitutive activation of one of the pro-growth pathways may be sufficient for the continued growth. Therefore, cancer cells fail to or only partially respond to starvation conditions and continue to promote growth instead of entering a protected mode.

factors, whereas cancer cells express oncogenes that can continue to relay the effect of growth factors even in their absence (Hanahan and Weinberg, 2000).

Such "independence" is in many cases caused by the autocrine production of growth factors, or by mutations that cause the constitutive activation of membrane receptors, or of intracellular signal transduction proteins. Some of the extracellular factors overproduced by cancer cells include PDGF, VEGF and IGF-I (Hanahan and Weinberg, 2000). Perhaps the most important contribution to the "growth factors independence of cancer cells" comes instead from intracellular alterations, particularly in genes coding for components of the Ras/Raf/MAPK and the PTEN/PI3K/AKT pathways. Altered forms of Ras that can render the cell growth independent from extracellular signals are found in approximately a quarter of all cancers (Medema and Bos, 1993) and half of colon cancers (Kinzler and Vogelstein, 1996). The interaction between Ras and PI3K may further stimulate growth and also activate anti-apoptotic pathways in the cancer cells (Castellano and Downward, 2011). Another highly relevant feature of cancer cells is the ability to "disobey" anti-growth orders. p53 or genes upstream or downstream of it, are well-conserved coordinators of stress and metabolic response which are inactivated in the great majority of cancers (Junttila and Evan, 2009; Vousden and Ryan, 2009). For example, mutations in the *MDM2-p53* and *Rb-E2F* pathways contribute to cancer progression by allowing malignant cells to override cell cycle check points (Polager and Ginsberg, 2009). The retinoblastoma protein (Rb) is one of the central negative regulators of proliferation in normal cells (Weinberg, 1995). PTEN, which negatively regulates the PI3K/AKT/mTOR pathway, is another tumor suppressor that is frequently inactivated in human cancers (Hollander et al., 2011).

The constant or at least frequent proliferation of cancer cells demands entirely different cellular metabolic requirements, since macronutrients are utilized to provide both cellular infrastructure as well as energy. For instance, the Warburg effect represents an important portion of such cellular reprogramming by shifting malignant cells to a more aerobic glycolysis mode that requires increased glucose consumption not resulting in oxidative phosphorylation; i.e., mitochondrial respiration does not increase proportionally to glucose uptake. It has been proposed that the excess glucose consumed by malignant cells is channeled through

alternate pathways with the purpose of producing the biomass (nucleotides, lipids, amino acids, etc.) required for successful cell division (Vander Heiden et al., 2009). We now understand that oncogenic mutations (e.g. PI3K/Akt and p53) do not simply promote mitogenesis, but, in fact, frequently regulate cellular metabolism (Vander Heiden et al., 2009). However, as discussed above, normal cells, unlike cancer cells, rapidly adapt to fasting, and shift the finite cellular energy to focus on cellular protection with the intention of maximizing the protection of its macromolecules and organelles and survival. Because severe starvation conditions have been frequently encountered by organisms ranging from bacteria to humans, normal cells of any type recognize the starvation environment and respond in a highly coordinated manner. Because down-regulation of proto-oncogenes plays such a central role in this starvation response, the oncogenes in cancer cells prevent this response (Fig. 3). This remarkable characteristic is fundamental in causing a fasting-dependent protection of normal but not malignant cells. In addition, fasting reduces nutrient availability, in particular that of glucose, which is the preferential carbon source for cancer cells, but also causes major changes in ketone bodies, insulin, IGF-I, IGFBP-1, and a variety of other molecules and factors which generates an environment detrimental to many cancer cell types (Lee et al., 2012).

## 5. Enhancing cancer treatment by fasting induced DSR: translational potential

Chemotherapy is the most widely adopted strategy for the treatment of a wide range of cancers (Chabner and Roberts, 2005) but the toxicity of the treatment makes it only partially effective particularly with advanced malignancies. As discussed earlier, fasting has been recently demonstrated to selectively protect normal cells and organisms from chemotherapy toxicity, while simultaneously sensitizing tumors (Raffaghello et al., 2008; Lee et al., 2012) (Fig. 4A–C). In the original study, different strains of mice were allowed to consume only water for 48–60 h (short-term starvation: STS) before treatment with high doses of etoposide, a widely used chemotherapeutic drug that damages DNA by multiple mechanisms and displays a generalized toxicity profile ranging from myelosuppression to liver and neurologic damage. Mice fed ad libitum, showed signs of etoposide-induced toxicity including reduced mobility, ruffled hair, and hunched back posture, whereas mice fasted prior to chemotherapy showed no visible signs of stress or pain (Raffaghello et al., 2008). In addition, 43% of control animals, and only one in the fasted group died from acute etoposide toxicity (Raffaghello et al., 2008). In order to evaluate the DSR against chemotherapy of normal and malignant cells, a metastatic model was established by intravenously injecting mice with neuroblastoma cells. Tumor-bearing mice were then fasted for 48-h prior to etoposide treatment. Fasting protected mice from etoposide without interfering with its killing of tumor cells (Raffaghello et al., 2008). Further, fasting has been recently shown to sensitize 15 of 17 cell lines tested to the widely used chemotherapy drug doxorubicin. Also, fasting alone retarded tumor progression, which was significantly enhanced when combined with chemotherapy in mouse models of various cancers (Lee et al., 2012).

During starvation, several changes in the pro-growth GH/IGF-I axis that optimize survival and maintenance under the new conditions occur. In particular, after a 72-h fast, mice lose approximately 20% of body weight, glucose levels are reduced by 41%, GH levels slightly increase, and IGF-I levels decrease by 70% (Lee et al., 2010). Moreover, the level of IGFBP-1, which regulates the bioavailability of IGF-I by sequestering it and avoiding its interaction with the IGF-I receptor, increases 11.4-fold in starved mice (Lee et al., 2010). Similarly to the results obtained in mice, in humans, glucose and IGF-I levels decrease dramatically in response to a 36–120-h fast despite increased GH secretion (Maccario et al., 2001; Merimee and Fineberg, 1974; Norrelund, 2005; Thissen et al., 1999).

As briefly mentioned earlier, the reduction of IGF-I has been demonstrated to be a mediator of the protection against chemotherapy toxicity in mice that underwent a 48-h fast (Lee et al., 2010). Analogously to starved mice, transgenic mice with a conditional liver *igf1* gene deletion (LID), that causes a postnatal 70–80% reduction of circulating IGF-I, display increased resistance to high-doses of various chemotherapeutic drugs such as cyclophosphamide (CP), 5-fluorouracil (5-FU) and doxorubicin (DXR) (Lee et al., 2010) (Fig. 4D–F). Experiments with LID mice intravenously injected with B16 melanoma cells and treated with two cycles of high-dose DXR, showed that decreased IGF-I not only protects the host against the toxicity of chemotherapy but also reduces tumor progression (Lee et al., 2010) (Fig. 4G). Notably, the IGF-I receptor (IGF-IR) is the target of many anti-cancer drugs since its constitutive activation is common in a variety of tumors (Pollak et al., 2004). For example, the down-regulation of IGF-IR results in mammary tumor regression in a majority of the mice without recurrence (Jones et al., 2010). Therefore, IGF-IR-targeting agents including small antagonists and antibodies have entered clinical trials for cancer patients (Gualberto and Pollak, 2009). Although it is still early to draw conclusions regarding efficacy of IGF-IR inhibitors, phase I studies reveal an acceptable safety profile, and recent evidence from a phase II studies suggests that co-administration of an anti-IGF-IR antibody with chemotherapy for non-small-cell lung cancer improves objective response rate and progression-free survival (Gualberto and Pollak, 2009). Notably, the major induction of the IGF-IR blocker IGFBP1 caused by fasting may provide a drug-like effect on the proliferation of IGF-IR-dependent tumors in addition to providing the other benefits described in this review.

A recent study describes 10 cases of patients affected by different types of tumors, ranging from stage II breast cancer, stage IV esophageal, prostate, to lung malignancies, who had voluntarily fasted prior to (48–140 h) and/or following (5–56 h) chemotherapy, under the supervision of their treating oncologists (Safdie et al., 2009) (Fig. 4H). None of these patients, who received different chemotherapy drugs in combination with fasting, reports significant side effects caused by fasting alone other than hunger and light-headedness. In addition, most of the chemotherapy-associated toxicities such as fatigue, weakness, and gastrointestinal side effects, are reported to be significantly reduced by the patients who underwent fasting (Safdie et al., 2009). It is noteworthy that, when it was possible to assess tumor progression by tumor volume or markers, fasting did not appear to interfere with the efficacy of chemotherapy (Safdie et al., 2009). Although these results suggest that fasting in combination with chemotherapy is safe and could reduce common side-effects associated to chemotherapy, only controlled randomized clinical trials can establish its full potential.

There are currently several clinical trials studying fasting in combination with chemotherapy in cancer patients, most of which are still ongoing or ready to recruit participants.

The first clinical trial on a form of fasting in combination with chemotherapy was sponsored by King Fahad Medical City in Riyadh, Saudi Arabia (ClinicalTrials.gov Identifier: NCT00757094), who evaluated the safety of fasting in 12 patients who received chemotherapy during the month of Ramadan. This study has been completed but the results are yet to be published. However, because the fasting period is much shorter (fasting every day but only during daylight hours) than that predicted by animal and preliminary human studies to be effective, and also because patients may tend to overeat to compensate for the short fast, the Ramadan-associated study is not likely to reveal major benefits provided by the altered diet. In July 2009, the University

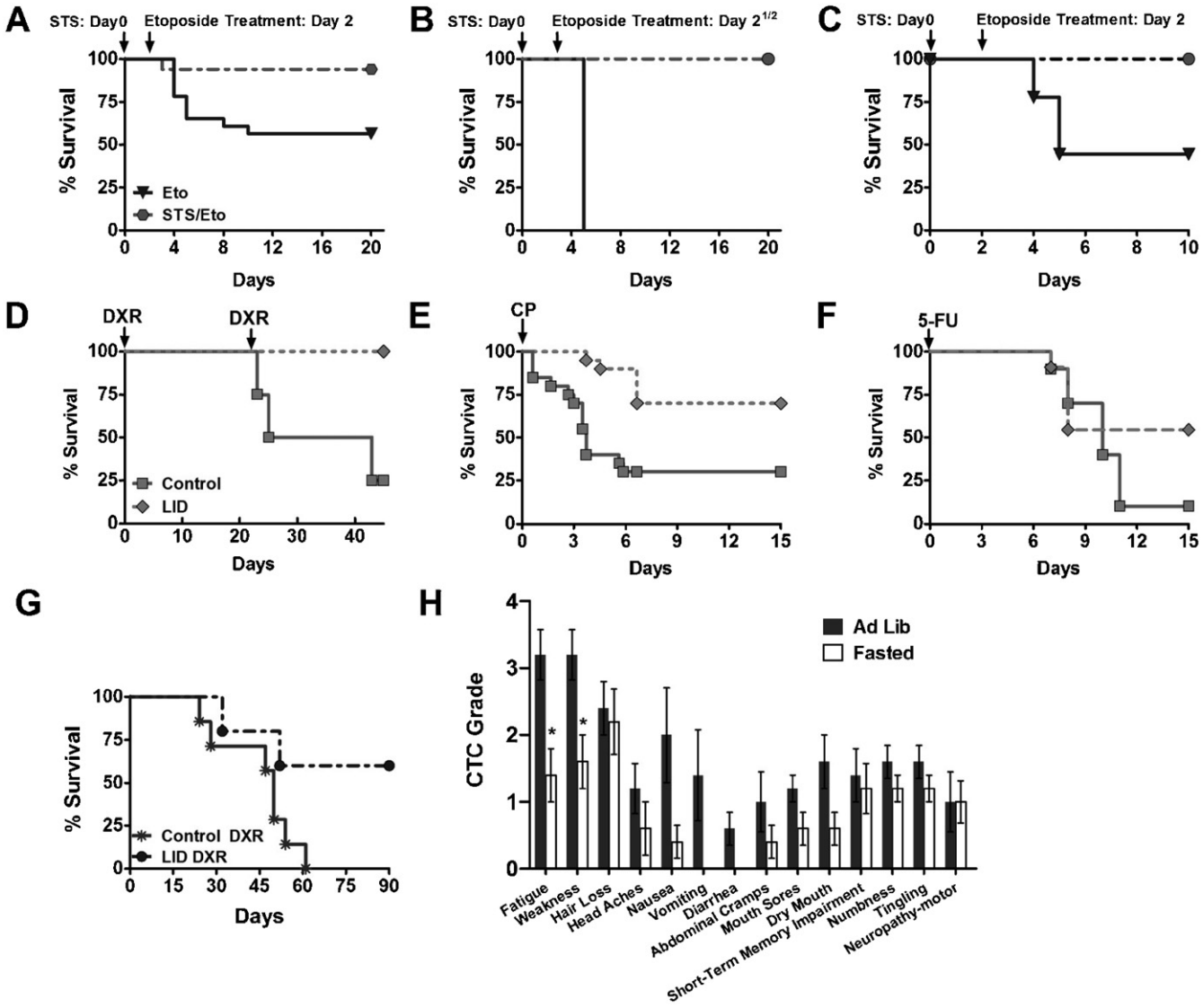


**Fig. 4.** Fasting selectively protects normal cells from chemotherapy toxicity. (A–C) Mice from 3 different genetic backgrounds were fasted for 48–60 h prior to high-dose etoposide administration. (A) A/J mice were fasted for 48 h. (B) CD-1 mice were fasted for 60 h prior to chemotherapy administration. (C) Athymic Nude mice were fasted for 48 h. (D–F) Transgenic mice with a conditional liver *igf1* gene deletion (LID) were treated with (D) 2 cycles of high-dose doxorubicin (DXR), (E) high-dose cyclophosphamide (CP), and (F) high-dose 5-FU. (G) LID mice bearing metastatic melanoma were treated with 2 cycles of high-dose DXR. (H) Patients self-reported side-effects after chemotherapy with or without fasting based on the common toxicity criteria (CTC) outlined by the National Cancer Institute (NCI).

of Southern California (USC)/Norris Comprehensive Cancer Center (ClinicalTrials.gov Identifier: NCT00936364) initiated a clinical trial to test the efficacy and safety of 24–72 h of fasting in combination with platinum-based chemotherapy in patients diagnosed with bladder cancer. A more recent extension will include patients affected by breast, ovarian and lung cancer. This trial is still ongoing. In August 2010, the Mayo Clinic Cancer Center (ClinicalTrials.gov Identifier: NCT01175837) started to enroll, and is still recruiting, patients older than 18 years who are affected by lymphomas and leukemias to fast in combination with chemotherapy. A clinical trial sponsored by Leiden University Medical Center, but not yet open for recruitment (ClinicalTrials.gov Identifier: NCT01304251) will instead study the effects of short-term fasting on tolerance to adjuvant chemotherapy in breast cancer.

Taken together, all the data discussed above suggest that fasting is safe and has the potential to be translated into clinical interventions for the protection of patients against chemotherapy-induced toxicity. Moreover, because of its effects on a variety of proteins and molecules, including IGF-I and glucose, and based on animal studies, fasting also has the potential to affect cancer progression with or without chemotherapy (Lee et al., 2012). However, more animal and cellular studies are needed to understand the role of fasting and of less extreme forms of DR on the differential protection of normal and cancer cells and on the mechanisms mediating these effects. The completion of clinical trials on fasting and cancer treatment will also be essential in understanding its clinical potential.

## 6. Conclusion

In the US, the death rate for heart disease has declined steadily between 1975 and 2007, but cancer death rates have been relatively constant (Siegel et al., 2011). Part of the limited efficacy of cancer treatment is due to the toxicity of chemotherapy to the host but also to the ability of cancer cells to become resistant to many toxic drugs. A variety of novel treatment options are being tested but it will take many years before these treatments are widely adopted and even those therapies which will be shown to be effective for

one set of patients or a specific tumor, may not be effective for others. Thus, in addition to more sophisticated and personalized cancer treatments, it is essential to develop treatments whose efficacy is as broad as that of the common chemotherapy drugs but far superior and with greatly reduced side effects. In addition, progress in this direction should not require decades but should attempt to find translational applications in the near future. The emerging understanding of the relationship between starvation/calorie restriction and stress resistance genes and pathways in eukaryotic cells has provided novel strategies to improve cancer treatment focused not only on the killing of tumor cells but also on the protection of normal cells and on how they respond differently to starvation conditions. Considering that MDR and reduced detoxification in cancer cells are recognized as major problems in cancer therapy, particularly in the attempt to achieve cures and not only modest extensions in survival, the understanding of the link between starvation conditions, specific nutrient restricted diets and multidrug resistance and other detoxification systems is undoubtedly important to maximize the differential protection of normal and cancer cells. If fasting affects MDR proteins differentially in normal and cancer cells, it may provide a therapy to reduce MDR and possibly the toxicity of MDR inhibitors.

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