American Institute of Integrative Oncology
RESEARCH & EDUCATION

Foundations of Integrative Oncology
COURSE

GRAND ROUNDS CALL
With Dr. Nalini Chilkov
November 14th, 2018
Second Wednesday of Every Month
5:30 PM Pacific / 6:30 PM Mountain / 7:30 PM Central / 8:30 PM Eastern

Clinical Pearl: Should Cancer Patients Fast? Risks and Benefits

See the attached slides

Case Study: 75yo F DCIS

Submitted by: Judy Pruzinsky

Overview: DCIS diagnosed September 2018. 7mm focus, no invasive cancer cells and margins are clean. Nuclear grade 3. Central comedonecrosis. Comedocarcinoma and cribriform growth patterns. 6 of 8 positive cores. Surgery on October 1 - may or may not have radiation based on results of Oncotype testing. Surgery resulted in clean margins.

Core Questions:
Patient believes she is cancer free (clean margins after surgery) and while she is on basic cancer protocol for now, she may not want to say on protocol for long. What else can you suggest?

Recommendations: See separate treatment plan notes.

Case Study: 61yo M Glioblastoma Multiforme

Submitted by: Stacy D'Andre

Overview: GBM left temporal lobe diagnosed May 2018. Offered chemo and radiation. Patient efused radiation. Treated with low dose temodar. Tumor grew back 3 months later and was resected. Currently on temodar and focal radiation. Methylation negative. Neuro deficits from surgery, poor memory and poor cognitive function.

Core Questions:
1. What would you suggest as a plan for overall maintenance of QOL and support of cognitive function?
2. What would you recommend for natural PARP inhibition for unmethylated GBM?

Recommendations: See separate treatment plan notes.

Questions & Answers

Carol Patti: **How do you deal with those patients you watch pass on?**

Dr. Chilkov's Response:
Death is not a failure. Death is the natural outcome of birth. If we can facilitate the process of patients with terminal illness to be one of healing such that they find meaning, are at peace and are able to have closure with loved ones, then we have made a significant contribution. Just as birth is sacred, so is the process of dying and taking one's last breath.

Resource:
Two books that I recommend by Joan Halifax, PhD.: *Being with Dying* and *Standing at the Edge*.

Carol Patti: **How would you recommend nourishing a patient when they get so sick from the treatments and even a protein shake makes them sick? I use bone broths, congee, ginger tea, lemon water, etc. - would love to know if you have any further recommendations - even on a supplement level that is not contraindicated.**

Dr. Chilkov's Response:

- **All patients should take anti-nausea medications s**o that they can maintain calorie and nutrient repletion
- Make sure patient is replete with fluids and electrolytes
- Best to ingest all WARM, cooked foods. Nothing raw or cold (prefer not to use congee-too high in CHO)
- Shakes can be room temperature, add digestive warming herbs such as ginger and cinnamon
- Bone broth is an excellent choice - minerals, 10g protein/cup, electrolytes (if made with vegetables)
- Can add nourishing and restorative herbs to the broth
    - Chinese medicinal mushrooms (Clinical Synergy Mycoceutics Immune Max powder),
    - Astragalus,
    - Red Panax Ginseng
    - Digestive herbs: Chinese Traditional formula Po Chai Pills, Curing Formula (Kang Ning Wan, Clinical Synergy Complete Digestive Balance)
    - Seaweeds
    - Extra fat for calories (coconut oil, organic ghee, olive oil)
    - Slippery Elm Powder Ulmus rubra (demulcent)
- Address microbiome with pre biotics (saccharomyces boulardii) and probiotics: broad spectrum such as KlarieTherbiotic Complete or LIVE Culture BIOK, or very concentrated high dose such as Designs for Health ProBiomed 250B 1 packet daily x 14 days
- Repair GI lining with L Glutamine 15-20g/day in divided doses and Vitamin A 10,000 iu
- Replete magnesium (Buffered Glycinate Chelate) and potassium (organic vegetable broth)
- Liquid B Complex (methylated form) Pure Encapsulations
- Acupuncture (St 36, Sp3, CV 14, CV12, CV 6, H7, Sp, 4, PC6, Kid 16, LI11, LI 4, Liv 3, Ear Shen Men),
- Moxabustion to St 36, CV12, CV6, K16=Crossing Point of Kidney Meridian and Chong Vessel)

If the digestive tract cannot easily accept any food consider:
Integrative Therapeutics Inc. Physicians Elemental Diet (Powder)
432g bag = 12 scoops 1800 kcal
1296g bag = 36 scoops 5300 kcal

Physicians' Elemental Diet contains a balanced blend of macronutrients fortified with essential vitamins, minerals, and electrolytes to assure comprehensive support as a sole source of nutritional intake for limited periods.

It has been specifically formulated to contain **free amino acids, partially hydrolyzed carbohydrate and medium chain triglycerides to aid in their absorption from the GI lumen.**

- Designed to maintain nutritional sustenance as a sole source of nutrition for up to four weeks.
- Produced as a strictly hypoallergenic formula, free from intact protein, polypeptides, corn, gluten, wheat, soy, and dairy.
- Formulated with a well-tolerated flavor for improved patient adherence.

https://www.integrativepro.com/Products/Gastrointestinal/Physicians-Elemental-Diet

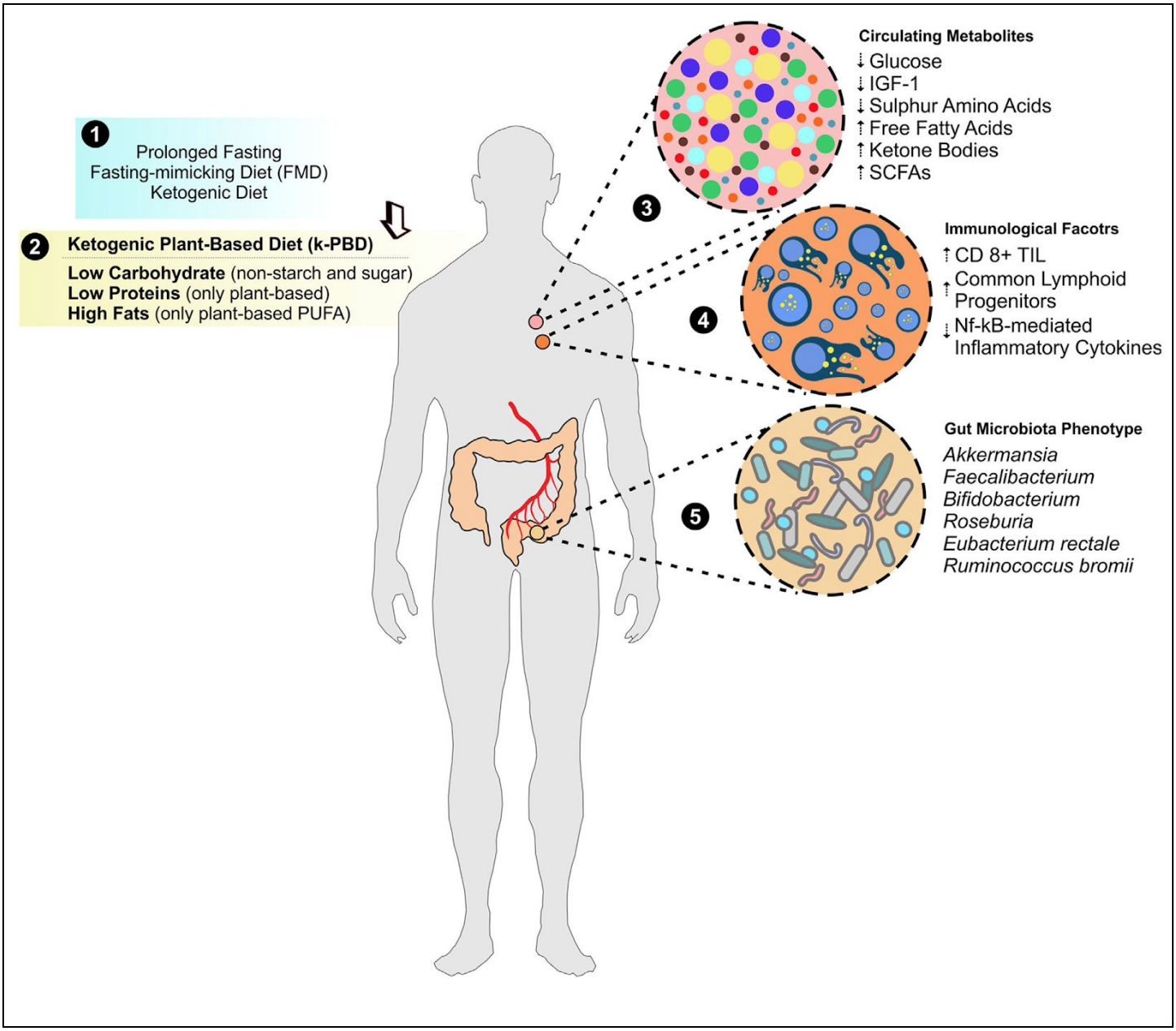
Research: Nutrient Modulation to Improve Cancer Therapy: *Pushing the Limits of Cancer Therapy: The Nutrient Game*

Lettieri-Barbato, D., & Aquilano, K. (2018). pushing the Limits of Cancer therapy: the nutrient Game. *Frontiers in oncology*, *8*.
doi: 10.3389/fonc.2018.00148

Frontiers in Oncology | www.frontiersin.org
HYPOTHESIS AND THEORY
May 2018 | Volume 8 | Article 148

The standard cancer treatments include chemotherapy, radiotherapy, or their combination, which are generally associated with a multitude of side effects ranging from discomfort to the development of secondary tumors and severe toxicity to multiple systems including immune system. ***Mounting evidence has highlighted that the fine tuning of nutrients may selectively sensitize cancer cells to conventional cancer therapies, while simultaneously protecting normal cells from their side effects. Nutrient modulation through diet also improves cancer immunesurveillance in a way that severe immunosuppression could be avoided or even the immune response or immune-based cancer therapies be potentiated also through patient microbiota remodeling***. Here, we review recent advances in cancer therapy focusing on the effects of adjuvant dietary interventions (e.g., ketogenic diets, fasting) on the metabolic pathways within cancer cells and tumor environment (e.g., microbiota, immune system, tumor microenvironment) that are involved in cancer progression and resistance as well as cancer cell death. Finally, based on the overall literature data, ***we designed a nutritional intervention consisting in a plant based moderate ketogenic diet*** that could be exploited for future preclinical research in cancer therapy.

Improving metabolites and immunological anticancer profile by k-PBD.
Evidence from prolonged fasting, fasting-mimicking diet (FMD), and ketogenic diet demonstrated a strong usefulness as adjuvants in cancer therapy (1). In this issue, we propose a moderate ketogenic plant-based diet (k-PBD), low in carbohydrates (starch and sugars in particular) and animal proteins (poor in sulfur amino acids and selenium) but rich in fats [mainly in vegetable polyunsaturated fatty acids (PUFA)] (2), which could strongly modulate circulating metabolites (3), immunological factors (4), and gut microbiota asset (5) that overall create a hostile environment to cancer cells.



Research:

Association of Frequency of Organic Food Consumption With Cancer Risk
Findings From the NutriNet-Sant. Prospective Cohort Study
Baudry, J., Assmann, K. E., Touvier, M., Allès, B., Seconda, L., Latino-Martel, P., ... & Kesse-Guyot, E. Association of frequency of organic food consumption with cancer risk: findings from the NutriNet-Santé prospective cohort study. *JAMA Internal Medicine*.
doi:10.1001/jamainternmed.2018.4357
Published Online October 22, 2018

Findings
In a population-based cohort study of 68 946 French adults, a significant reduction in the risk of cancer was observed among high consumers of organic food.

Results
High organic food scores were inversely associated with the overall risk of cancer (hazard ratio for

quartile 4 vs quartile 1, 0.75; 95%CI, 0.63-0.88; P for trend = .001; absolute risk reduction, 0.6%; hazard ratio for a 5-point increase, 0.92; 95%CI, 0.88-0.96).

CONCLUSIONS AND RELEVANCE

A higher frequency of organic food consumption was associated with a reduced risk of cancer. Although the study findings need to be confirmed, promoting organic food consumption in the general population could be a promising preventive strategy against cancer.

Modulating the Effects of Chemotherapy by Fasting

TOXICITY IN CHEMOTHERAPY
WHEN LESS IS MORE

NEJM 366;24 nejm.org june 14, 2012

Chemotherapy Induced Oxidative Stress

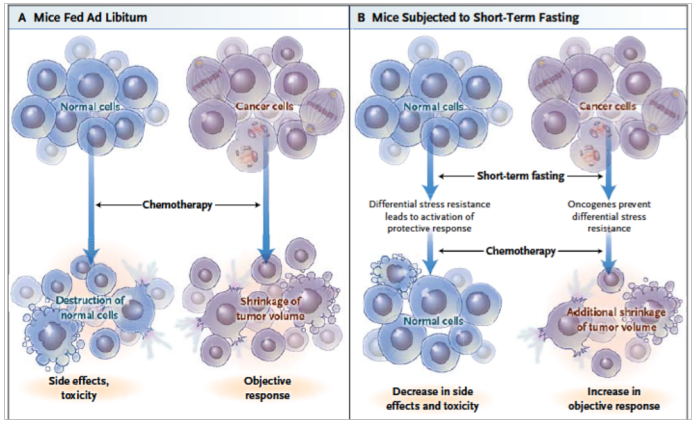
- Chemotherapy-induced oxidative stress reduces the rates of both the proliferation and the survival of cancer cells.
- It yields an objective response that can be quantified on the basis of shrinkage of the tumor volume
- Chemotherapy also affects normal cells, leading to toxic side effects

Chemotherapy Induced Oxidative Stress

- Short-term fasting before or after chemotherapy, or at both times, induces differential stress resistance in normal and cancer cells
- **In normal cells, fasting activates protective metabolic pathways that confer resistance to oxidative stress**
- **Cells with activated oncogenes are unable to turn on the protective response and thus remain sensitive to oxidative stress**.

Chemotherapy Induced Oxidative Stress

- **Fasting specifically augments levels of oxidative stress and sensitivity to oxidative damage inflicted by chemotherapeutic agents in cancer cells**
- These effects are accompanied by DNA damage and apoptosis



The Effect of Short-term Fasting on Chemotherapy-Associated Toxicity

- **Enhances responsiveness to and compliance with anticancer therapies.**
- **Potentiates effects of chemotherapy**
- **Reduces adverse effects**
  - **Fatigue**
  - **Weakness**
  - **Gastrointestinal Side Effects**
- **Extends Survival**

CAUTION: AGE MATTERS

Longo VD, et al

Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population.

Cell Metab. 2014 Mar 4;19(3):407-17

Fontana L, Partridge L, Longo VD. **Extending healthy life span — from yeast to humans.** Science 2010;328:321-6.

Lee C, Raffaghello L, Brandhorst S, et al. **Fasting cycles retard growth of tumors and sensitize a range of cancer cell types to chemotherapy.** Sci Transl Med 2012;4:124ra27.

Temel JS, Greer JA, Muzikansky A, et al**. Early palliative care or patients with metastatic non–small-cell lung cancer**. N Engl J Med 2010;363:733-42.

Safdie FM, Dorff T, Quinn D, et al. **Fasting and cancer treatment in humans: a case series report**. Aging (Albany NY) 2009; 1:988-1007.

Laviano A, Seelaender M, Sanchez-Lara K, Gioulbasanis I, Molfino A, Rossi Fanelli F. **Beyond anorexia-cachexia: nutrition and modulation of cancer patients' metabolism: supplementary, complementary or alternative anti-neoplastic** Pharmacol 2011;668:Suppl:S87-S90.

Is There a Role for Carbohydrate Restriction in the Treatment and Prevention of Cancer?

Klement and Kämmerer Nutrition & Metabolism 2011, 8:75
http://www.nutritionandmetabolism.com/content/8/1/75

Carbohydrate Restriction Lower Plasma Insulin Levels

Reduces Carcinogenesis and Tumorogenesis

Delays Tumor growth

Increases Tumor Latency

High insulin and IGF1 levels accelerate proliferation and progression towards a more aggressive, glycolytic phenotype in cancer cells

Ketone bodies to help in cancer prevention through their ability to protect the mitochondria from inflammation and ROS

Lee C, Raffaghello L, Brandhorst S, et al. **Fasting cycles retard growth of tumors and sensitize a range of cancer cell types to chemotherapy**. Sci Transl Med 2012;4:124ra27

Low Glycemic CHO Restricted Diets and Cancer Development

Most, if not all, **tumor cells have a high demand on glucose** compared to benign cells of the same tissue and conduct glycolysis even in the presence of oxygen **(the Warburg effect).**

In addition, **many cancer cells express insulin receptors (IRs) and show hyperactivation of the IGF1R-IR pathway**.

Evidence exists that **chronically elevated blood glucose, insulin and IGF1 levels facilitate tumorigenesis and worsen the outcome in cancer patients.**

Low Glycemic CHO Restricted Diets and Cancer Development

The involvement of the glucose-insulin axis may also explain the **association of the metabolic syndrome with an increased risk for several cancers.**

CHO restriction has already been shown to exert favorable effects in patients with the metabolic syndrome.

Epidemiological and anthropological studies indicate that restricting dietary CHOs could be beneficial in decreasing cancer risk.

Low Glycemic CHO Restricted Diets and Cancer Development

Many cancer patients, in particular those with advanced stages of the disease, exhibit altered whole-body metabolism marked by

increased plasma levels of inflammatory molecules, impaired glycogen synthesis, increased proteolysis and increased fat utilization in muscle tissue, increased lipolysis in adipose tissue & increased gluconeogenesis by the liver.

High fat, low CHO diets aim at accounting for these metabolic alterations. Studies conducted so far have shown that such diets are safe and likely beneficial, in particular for advanced stage cancer patients.

Low Glycemic CHO Restricted Diets and Cancer Development

CHO restriction mimics the metabolic state of calorie restriction or - in the case of KDs - fasting.

The beneficial effects of calorie restriction and fasting on cancer risk and progression are well established.

CHO restriction thus opens the possibility to target the same underlying mechanisms without the side-effects of hunger and weight loss.

Low Glycemic CHO Restricted Diets and Cancer Development

Some laboratory studies indicate a direct anti-tumor potential of ketone bodies.

During the past years, a multitude of mouse studies indeed proved anti-tumor effects of KDs for various tumor types, and a few case reports and pre-clinical studies obtained promising results in cancer patients as well. Several registered clinical trials are going to investigate the case for a KD as a supportive therapeutic option in oncology.

Klement and Kämmerer Nutrition & Metabolism 2011, 8:75
http://www.nutritionandmetabolism.com/content/8/1/75
Is there a role for carbohydrate restriction in the treatment and prevention of cancer?

Pushing the Limits of Cancer Therapy: The Nutrient Game

Daniele Lettieri-Barbato and Katia Aquilano
Frontiers in Oncology May 2018 | Volume 8 | Article 148

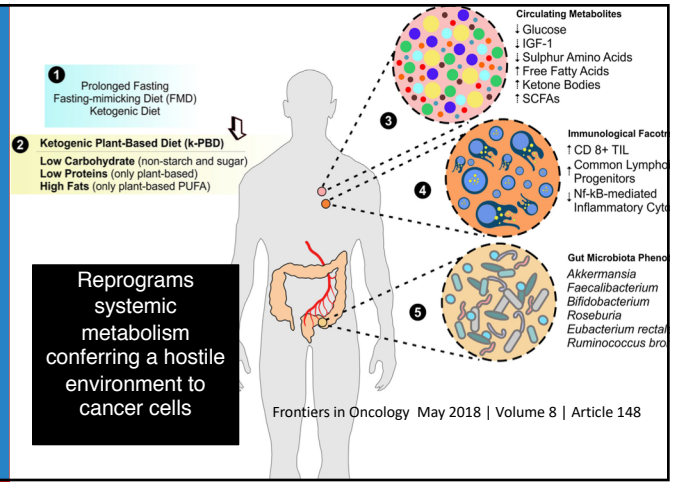
Moderate ketogenic plant-based diet (k-PBD) creates a hostile environment to cancer cells

Low in carbohydrates (starch and sugars in particular) and animal proteins (poor in sulfur amino acids and selenium)

Rich in fats [mainly in vegetable polyunsaturated fatty acids (PUFA)]

- Strongly modulates
- Circulating metabolites
- Immunological factors
- Gut microbiota (increase Akemansia
- Intestinal short chain fatty acids

Improving metabolites and immunological anticancer profile by Ketogenic Plant Based Diet



Frontiers in Oncology May 2018 | Volume 8 | Article 148

American Institute of Integrative Oncology
RESEARCH & EDUCATION

CASE STUDY SUBMISSION

Important: In observance of HIPAA and the sacred trust between care giver and patient, absolutely no patient names or identifying information is to be disclosed. Patient privacy is to be preserved. If you attach any medical records, pathology, surgical or laboratory reports, all names are to be removed.

Date	October 12, 2018
Clinician Name & Credentials	Judy Pruzinsky, L.Ac.
Email	judy@judypruzinsky.com

Describe Your Patient (Please SUMMARIZE and use economy of words. You will have 15 minutes to present)

Age, Gender & Ethnicity	76 yr old woman, Irish
Body Type	medium height, slightly obese
Values *What is most important to this patient? (Quality of Life, Decision Making, Side Effects?)*	
Stress Resilience	Extremely capable, can push through, though with age is starting to feel it and know it is not what she wants
Other	
Primary Diagnosis & Date *(ex. Breast Cancer L, T3 N1 M0, BRCA1 positive, grade 3, Ki67 > 45%)*	September 2018 DCIS - 7 mm focus, and no invasive cancer cells, all margins are clean Nuclear grade 3 Necrosis: central comedonecrosis Architectural pattern: comedocarcinoma and cribriform growth patterns # of positive cores: six of eight cores
Secondary Diagnosis *(ex. Diabetes Type 2, Obesity)*	Sl obesity ER positive; H score: 300, suspect insulin resistant, Dx of hiatal hernia with bleeding and subsequent anemia, goes for iron infusion every 6 months or so With current flurry of doctor visits was put on blood pressure meds, expected temporary with lifestyle changes and weight reduction

Patient Status

☐ New Diagnosis ☐ Recurrence ☐ In Treatment ☐ In Recovery ☐ In Remission ☐ At Risk

Concomitant and/or Complicating Factors *(ex: poorly controlled diabetes, insomnia, poor support system)*	Sleep moderate apnea, awaiting machine is good
Adverse Effects of Cancer or Cancer Treatments *(ex. anxiety-depression, diarrhea, peripheral neuropathy)*	
Relevant Laboratory, Pathology & Medical Reports *(attach a PDF with patient identifying information removed or summarize)*	

Brief Summary of Recent History

Endometriosis, Hysterectomy (1980) on HRT until recent dx.

Hip surgery July 2018

Brief Summary of Additional Relevant Health, Medical, Psycho-Social and/or Family History

Other Relevant Information

Such as Chinese or Ayurvedic diagnosis, Naturopathic/Homeopathic Information, etc. *(ex. Liver Qi Stagnation, Dysbiosis)*

Heart - Kidney (Shao-yin) deficiency, can get very fatigued (from years of pushing) does respond well to acupuncture.

Brief Summary of Relevant Past Oncology or Medical Treatments

(ex. surgery, radiotherapy, chemotherapy, immunotherapy, hormone therapy, drug therapy)

Surgery Oct 1

Dependent on results from Oncotype testing; may or may not have radiation

Summary of Recent and Current Treatments

Medical Oncology Care *(surgery, radiotherapy, chemotherapy, immunotherapy, hormone therapy, drug therapy)*

Integrative Oncology Care *(nutraceutical, botanical, phytochemical, acupuncture, energy medicine, other)*

Comes every week (to 2 weeks) for acupuncture
Every 2 weeks + for chiropractice

Your 2 Core Questions (stated clearly and succinctly)

1. Since surgery went well, clean margins.. thinks she is cancer free. I explained wanting to be clear of all micro growths, not seen through testing. I have also, before dx and now, explained the importance of inflammation and oxidative stress. For now she's on basic cancer protocol (though not having shakes as she really doesn't like taste). I can foresee her not wanting to stay on protocol long because of feeling "cancer free." Do you suggest anything else?
2.

Attached Medical Records for Reference (with patient identifying information removed)

PROPOSED TREATMENT PLAN Your case will not be reviewed without a completed proposed treatment plan

Nutriceutical, Phytochemical and Botanical Supplements (name of supplement, dosing)

Foundation Nutrition Supplements:
DFH Multi- vitamin/mineral copper and iron free
Omega 3 Fatty Acids, up to 4g / day
Vit D and K , 2-10,000 IUs
Probiotic, 15 billion 1-2/day
Melatonin, 10-20 mg day
PaleoFiber start 1 tsp 2/day, work up to 1 Tbsp 2/day

Targeted Supplements:
Broccoprotect 2/day
EGCg, 500 mg BID
Curcum-Evail, 2-3 grams/day
Immune + 4 3/day
CA Support 4 3/day
Berberine – 1/day
Milk Thistle 1 cap = 140 mg 2-4 caps/day (may change to Hepatatone)
For the first month post surgery
Glutamine 1 tsp with breakfast and dinner

Functional Foods and/or Therapeutic Shake

Re lowering beta-glucuronidase to lowering estrogen exposure:
Cultured non dairy products, cruciferous vegetables, garlic and onion family, probiotics (Probiophage), prebiotics (Paleofiber).

Dietary Guidelines

History of not eating well, not like to cook and not like shakes
With diagnosis a couple weeks ago she has finally changed to low glycemic, non-dairy, gluten-free, more vegetables, good protein and fats.
Gave up coffee and wine

Lifestyle Guidelines

Is starting back to pilates 2/week, and at home bicycle

Recommended Diagnostics

Referrals to specialists

Other Notes (please do not include additional notes in your email – notate them here within the case study)

Case Study

ER+ DCIS Resolution and Control 76 yo. Irish heritage
ER+ DCIS-option to do nothing: Studies show DCIS often resolves on its own However, this patient has a tumor microenvironment that is supportive of proliferation

TCM Heart-Kidney Shao Yin deficiency

CC September 2018 ER + DCIS - 7 mm focus, and no invasive cancer cells, all margins are clean Nuclear grade 3
Necrosis: central comedonecrosis
Architectural pattern: comedocarcinoma and cribriform growth patterns
# of positive cores: six of eight cores

Surgery-clean margins

Additional Problems
Obese
Insulin resistance
Hypertension
Sleep Apnea (obesity, glycemic control, cortisol balance, hypertension, immune compromise)
Hiatal hernia
Poor nutritional status

PLUS Consider Age related:
Cognitive Health
Bone Health

Hx of
HRT
Endometriosis (estrogen, inflammation),
Hip Replacement (due to arthritis or osteoporosis??)

<u>Foundation+Targeted Supplements</u>
ITI ProThriver Wellness Muti 1/2x/day (Boron, Fe, Cu free)
DFH Vitamin D Supreme 5000iu. 1/2x/day
DFH Osteoben 2/2x/day
Klaire Target gbx 1packet daily
DFH Buffered Magnesium Chelate 2/2x/day or you titrated dose
DFH Curcumevail 2/2x/day

DFH EGCG 2/2x/day
DFH Omegavail TG 1000 2/2x/day.
DFH Broccoprotect (or Hormone Protect) 1/2x/day
DFH Annatto Tocotrienols 2/2x/day
Clinical Synergy Pure Honokiol 2/2x/day and 2 at bedtime
Clinical Synergy Mycoceutics Immune Max
Powder 1 scoop twice daily or
Capsules 6 caps twice daily
PUR Hawthorne Berry caps. (Hypertension-mild beta blocker)
DFH Taurine 2 bid (Hypertension)
VN Pancreatin and Ox Bile Digestive enzymes with meals (consdier adding HcL)

98% Pure Resveratrol 1/2 level teaspoon day (2.5 grams)

Bedtime
Vital Nutrients Melatonin 10mg
Clinical Synergy Pure Honokiol 2 caps

Custom Tonic #1 : Bone Health+Brain Health 2 teaspoons daily
Shake well Mix with warm water or tea

16 oz	8oz	
480ml	240ml	
180	90	Restore Right
60	30	Epimedium
30	15	Psoralea
30	15	Fr Cornii
60	30	White Peony
30	15	Bacopa
30	15	Gotu Kola
40	20	Ginkgo
20	10	Licorice Root

Custom Tonic #2 Tumor Control + Stress Reslience Formula
2 teaspoons daily Shake well Mix with warm water or tea

16oz	**8 oz**	
480ml	240ml	
50	25	Scutellaria barbata
50	25	Scutellaria baicalensis
40	20	Polygonatum Soloman's Seal
40	20	Red Sage Dan Shen Salvia milt.

40	20	Green Tea
40	20	Oldenlandia
40	20	Urtica Urens Nettle Root
40	20	Feverfew
30	15	Ganoderma
30	15	Ashwaganda
30	15	Astragalus
20	10	Chen Pi Tangerine Peel
20	10	Schizandra
10	05	Licorice Root

Intermittent Fasting 13+ hours no calories. (dinner>>>next breakfast)
OutSmart Cancer Diet-Modified Paleo-Keto (work with our nutritionist)
Low Carb, Low Sugar, Low Starch, Anti-Inflammatory
Healthy Fats and Oils
Rainbow of colors: vegetables that grow above ground,
Limit fruit to 1 cup berries daily 60 grams+ protein daily

Intravenous High Dose Vitamin C

Screening:
CANCER TERRAIN LABS-

LIFESTYLE
Functional Nutrition Coaching
Exercise: Minimum 30 min+ moderate exercise daily (Sweat), weights (improve insulin resistance, bone and brain health)
Sleep 7-9 hours nightly
Meditation, Prayer, Visualization
Skin Dry Brushing to stimulate lymphatic drainage

Two 14 day cycles in the next 6 months

CYTOTOXIC 10-14 DAY CYCLE

CYTOTOXIC HERBAL THERAPY PLUS IV THERAPY

Combine protocol below concurrently with IV Vit C (a cytotoxic therapy)

Cytotoxic Botanicals

- **CytoToxic Compound- for 14 days x 2 cycles**

1/2 teaspoon 3x/day diluted in warm water or tea with food or shake
(Suspend regular tonics during this time)

40 ml Polygonatum (Solomon's seal) root
20 ml Taxus brevifolia (Yew) tips
20 ml Catharanthus (Madagascar Periwinkle) Leaf
40 Phyto Cyto (Asimina triloba, Taxus brevifolia, Catharanthus rosea, Viscum album, Phytolacca americana, Podophyllum pelatum)

PLUS

- ARG Super Artemesinin 3 caps twice daily for 10 days once per season

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Date	10/16/18
Clinician Name & Credentials	Stacy D'Andre
Email	dandrs1@sutterhealth.org

Describe Your Patient (Please SUMMARIZE and use economy of words. You will have 15 minutes to present)

Age, Gender & Ethnicity	61 yo Hispanic male
Body Type	normal BMI
Values *What is most important to this patient? (Quality of Life, Decision Making, Side Effects?)*	QOL, side effects
Stress Resilience	not great due to illness, wife very anxious
Other	
Primary Diagnosis & Date *(ex. Breast Cancer L, T3 N1 M0, BRCA1 positive, grade 3, Ki67 > 45%)*	Glioblastoma multiforme left temporal lobe dx 5/18, unmethylated (poor prognosis)
Secondary Diagnosis *(ex. Diabetes Type 2, Obesity)*	healthy otherwise

Patient Status

☐ New Diagnosis ☐ Recurrence ☑ In Treatment ☐ In Recovery ☐ In Remission ☐ At Risk

Concomitant and/or Complicating Factors *(ex: poorly controlled diabetes, insomnia, poor support system)*	Neuro deficits from surgery, poor memory/poor cognitive function, some depression and anger
Adverse Effects of Cancer or Cancer Treatments *(ex. anxiety-depression, diarrhea, peripheral neuropathy)*	fatigue
Relevant Laboratory, Pathology & Medical Reports *(attach a PDF with patient identifying information removed or summarize)*	attached file

Brief Summary of Recent History

Patient presented with headaches in May 2018. Imaging showed left temporal mass, resected showing GBM. He was offered chemo rads but refused rads. He went ahead with low dose temodar (usually given with radiation). 3 months later tumor grew back and it was re-resected. Currrently on temodar and focal radiation. Methylation negative.

Brief Summary of Additional Relevant Health, Medical, Psycho-Social and/or Family History

He is healthy otherwise, retired police officer. He is resigned to poor prognosis but his wife is very anxious and aggressive; he is much more laid back but they do argue a lot at home. Some of it due to poor memory/cognitive function

Other Relevant Information

Such as Chinese or Ayurvedic diagnosis, Naturopathic/Homeopathic Information, etc. *(ex. Liver Qi Stagnation, Dysbiosis)*

Naturopath started some supplements

Brief Summary of Relevant Past Oncology or Medical Treatments

(ex. surgery, radiotherapy, chemotherapy, immunotherapy, hormone therapy, drug therapy)

Surgery 5/18
Surgery re-resect 8/18
on temodar 75 mg/m2 with radiation currently

Summary of Recent and Current Treatments

Medical Oncology Care *(surgery, radiotherapy, chemotherapy, immunotherapy, hormone therapy, drug therapy)*

considering optune TTF

Integrative Oncology Care *(nutraceutical, botanical, phytochemical, acupuncture, energy medicine, other)*

Fish oil 3.5g /day, curcumin 3 g/d, resveratrol 2 tabs per day

Your 2 Core Questions (stated clearly and succinctly)

1. Plan for overall maintenance of QOL, support cognitive function

2. Natural PARP inhibition for unmethylated GBM

Attached Medical Records for Reference (with patient identifying information removed)

PROPOSED TREATMENT PLAN Your case will not be reviewed without a completed proposed treatment plan

Nutriceutical, Phytochemical and Botanical Supplements (name of supplement, dosing)

Foundation Nutrition Supplements:

MVI
magnesium
Green tea 5-10 cups per day
Already on Klaire probiotic/FO/curcumin, resveratrol

Targeted Supplements:

added quercetin as PARP inhib 500 mg BID
Added Lion's mane 2 g/d
Added melatonin 10-20 mg /d
Added CBD: THC 1:1 6-8 drops BID
Boswellia for edema 4 g/day

Functional Foods and/or Therapeutic Shake

Dietary Guidelines

KETO diet, glutamine restricted

Lifestyle Guidelines

Stress reduction and meditation
Walking/exercise daily
Counseling

Recommended Diagnostics

Referrals to specialists

Referral to counselor

Other Notes (please do not include additional notes in your email – notate them here within the case study)

November 2018

CASE STUDY Glioblastoma multiforme
Submitted by Dr. Stacy D Andre
61 yo Hispanic male retired police officer
Glioblastoma multiforme: L Temporal lobe. Unmethylated (poor prognosis)

Dx 05/2018.
Post surgical resection>>>Neurologic deficits, cognitive, memory, mood dysregulation, depression, anger, fatigue
Undergoing treatment with CT low dose temodar>>recurrence within 3 months>resected, declined RT orignally..with recurrence on LD Temodar + focal RT
Poor stress resilience
Wife very anxious..conflict at home
Considering Optune TTF

GOALS QOL SE Management

Current Supplements
Fish oil 3.5g.day increase to 6g
Curcumin 3 g/day increase to 6g
Resveratrol 2 caps daily. Up to 5g/day (98% pure powder level tsp ~ 5g)
Klaire Probiotic (Target gbx 1 packet daily)

Proposed tx plan
MVI. (copper free-iron free)
magnesium. (Mg threonate + Mg Glycinate 600mg/day)
Green tea 5-10 cups per day. (EGCG 4 g/day)

added
quercetin as PARP inhib 500 mg BID
Natural PARP inhibition for unmethylated GBM. (Niacinamide 2 g/day?)
Added Lion's mane 2 g/d (4 g/day)
Added melatonin 10-20 mg /d
Added CBD: THC 1:1 6-8 drops BID
Boswellia for edema 4 g/day

Ketogenic Diet
Stress Reduction
Walking-Exercise daily
Counselling
(Meditation-Prayer?)

Dr. Chilkov: Additional considerations

Cognitive Function
Bacopa, Gotu Kola Gingko,
Acetyl LCarnitine 2g
Mitochondrial Support. DFH Mitochondrial NRG 2/2x/day

Immunity-Adaptogens
Astragalus, Ganoderma
Vitamin D

Therapeutic Shake (ketogenic) NO FRUIT
Take with 2 caps
DFH Hydrolyzyme (protease)
and
ITI Lipase 1-2 caps

DFH ORGANIC PURE PEA PROTEIN 2 scoops 20 grams. 3 scoops 30 grams
1 heaping teaspoon hemp seeds (essential fatty acids support normal neurologic fx)
1 teaspoon chia seeds (essential fatty acids support normal neurologic fx)
1/2 avocado (essential fatty acids and vitamins)
1 heaping teaspoon coconut oil (essential fatty acids and medium chain triglycerides)
MRL Coriolus 1 scoop (polysaccharides and beta glucans for supporting immune modulation and control of inflammation)
DFH Paleo Reds 1 teaspoon (combination of red and purple fruits and berries in a concentrate to supply antioxidant phytochemicals)
*VN Acetyl L Carnitine powder 1/2 teaspoon
*DFH Phosphatidyl Choline Powder 1 heaping teaspoon
*DFH Phosphatidyl Serine powder 1/2 teaspoon
Natura Botanical Treasures Powder 1 teaspoon
MCT Oil (MCT COLADA) 1 tablespoon
½ avocado
Coconut milk or water unsweetened
vanilla, cinnamon, cardamom, mint leaves green tea leaves, orange zest, cacao nibs

Significant Lab Results
High NLR 8.0: 0.7=11.4 Very Poor Prognosis
Low Albumin 2.8 Poor Prognosis

NOTES from Donnie Yance on GBM

Brain tumors/radio-potentiation:
Pure Encapsulations **St. Johns Wort** (Hypericum) Capsules 600mg 4x/day
DFH **L-Taurine** 1000mg 1 cap 4x/day

DFH **Annatto Tocotrienols with Black Cumin Seed Oil** 2/2x/day

GLA: Vital Nutrients Borage Oil (500mg per cap) 1/4x/day
Clinical Synergy **Pure Honokiol** 2/4x/day

Chemotherapy: Little or no benefit from Temador
Other drug treatments to consider:
Selinexor
PARP Inhibitors; **Olaparib (Lynparza)**
Combine
High dose Tamoxifen + Navelbine (Vinorelbine) + Irinotecan
or
High Dose Tamoxifen + Iressa (Gefitnib)

Screen for Cytomegalovirus: If positive treat with Valgancyclovir

PARP Inhibition
- Green Tea DFH **EGCG** 2/4x/day
- Curcumin DFH **CurcumAvail** 2/4x/day
- (For high dose Green tea and Curcumin=
- NAT **Botanical Treasures** Powder -1 tsp 2x/day or caps 4/4x/day)
- Douglas Labs **Niacinamide** 500mg 4x/day

Additional Anti tumor Herb extracts
Oldenlandia (ursolic acid)
Saliva Milthiorhizza
Camptotheca (TOPO1)
PhytoCyto

AMERICAN INSTITUTE OF INTEGRATIVE ONCOLOGY
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The Names of Targeted Therapies

Give Clues to How They Work

Identifying the source, target, and mechanism of action by uncoding the generic names of targeted therapies. Each generic name gives information on the what, how, and where of each particular drug.

In contrast to traditional chemotherapeutic agents that affect rapidly dividing cells, targeted agents are more precise in the way they fight cancer. Presently**, two main families of targeted therapies exist—monoclonal antibodies and small molecule inhibitors**. The ending letters (stem) of the generic names are like surnames that tell what family the drug is from and how the drug works to kill cancer cells.

Monoclonal antibodies end with the stem "-mab" and small molecule inhibitors end with the stem "-ib". The "-mab" family of targeted therapies has three distinct methods for interfering with cancer cell growth.

1. Attach to receptors on the outside of cells to prevent the receptors from interacting with signaling molecules (e.g., growth factor receptors and growth factor interaction)
2. Deliver radioactive molecules or toxins to the inside of the cells through attachment to cellular receptors
3. Activate the body's natural immune response

The "-mab" family is used when receptor targets are overexpressed on the outside of cancer cells. Conversely, the "-ib" family targets processes within the cell and therefore must be small enough in molecular weight to enter the cell and interfere with proteins on both the inside and outside of the cell. Proteins that code for growth or inhibit growth are some of the targets of these small but powerful family of drugs.

The sub stem of the generic names of the "-mabs" identifies the source where the antibodies were generated or cloned. The three most common sources are

1. Chimeric human-mouse—drugs ending in "-ximab" (i.e., rituximab)
2. Humanized mouse—drugs ending in "-zumab" (i.e., bevacizumab)

3. Fully human—drugs ending in “-mumab” (i.e., ipilimumab).

Finally, both “-mabs” and “-ibs” contain an additional stem to describe the targeted therapies bullseye. For example, the “tu” in rituximab indicates the target is the tumor, the “ci” in bevacizumab designates the circulatory system, and the “li” in ipilimumab identifies the immune system target. Some of the intracellular targets for the “-ibs” include:

1. Tyrosine kinase inhibition—sub stem “-tinib” (i.e., imatinib)
2. Proteasome inhibition—“-zomib” (i.e., bortezomib)
3. Cyclin-dependent kinase inhibition—“-ciclib” (i.e., seliciclib)

The prefix of the generic names and the drug market names are where researchers and pharmaceutical companies—like the parents of the young dancers—take creative liberty.

The development of targeted therapies is expected to accelerate as new targets are identified, as a result, oncology nurses will need to stay up to date on the new medications so they can educate their patients on the way these therapies work as well as the possible side effects of the medications. Unfortunately, many people may have the idea that there are few if any side effects associated with targeted therapy. Although side effects can be less than those of standard chemotherapy, targeted therapies also affect normal cells to some degree.

RESOURCES

Chemotherapy and Biotherapy SIG.

Understanding Targeted Cancer Therapies
presented by the National Institute of Health.

Source: https://voice.ons.org/news-and-views/the-names-of-targeted-therapies-give-clues-to-how-they-work

frontiers in Oncology

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Pushing the Limits of Cancer Therapy: The Nutrient Game

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The standard cancer treatments include chemotherapy, radiotherapy, or their combination, which are generally associated with a multitude of side effects ranging from discomfort to the development of secondary tumors and severe toxicity to multiple systems including immune system. Mounting evidence has highlighted that the fine-tuning of nutrients may selectively sensitize cancer cells to conventional cancer therapies, while simultaneously protecting normal cells from their side effects. Nutrient modulation through diet also improves cancer immunesurveillance in a way that severe immunosuppression could be avoided or even the immune response or immune-based cancer therapies be potentiated also through patient microbiota remodeling. Here, we review recent advances in cancer therapy focusing on the effects of adjuvant dietary interventions (e.g., ketogenic diets, fasting) on the metabolic pathways within cancer cells and tumor environment (e.g., microbiota, immune system, tumor microenvironment) that are involved in cancer progression and resistance as well as cancer cell death. Finally, based on the overall literature data, we designed a nutritional intervention consisting in a plant-based moderate ketogenic diet that could be exploited for future preclinical research in cancer therapy.

Keywords: mitochondria, diet, fasting, immunemodulation, microbiota and immunity

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AN OVERVIEW ON THE CONTROL OF TUMOR PROGRESSION BY DIETARY INTERVENTIONS

A plethora of epidemiological and experimental data demonstrated the efficacy of geroprotective dietary regimens (e.g., fasting, calorie, proteins, or single amino acids restrictions) in cancer prevention (1–3). Furthermore, such dietary patterns are emerging to be effective in selectively killing cancer cells, whereas increasing resistance of normal cells to the toxic effects of the anticancer therapeutics.

Calorie restriction (CR), defined as 30–60% less of daily calorie requirement without malnutrition, is known to extend healthy life span from yeast to mammals (4). The anticancer effects of CR are known since several years (5). CR is particularly effective in reducing the incidence, mass, and metastasis of breast cancer cells (6, 7). Remarkably, applying CR in combination with radiotherapy enhanced the radiotherapy efficacy inducing a more pronounced apoptosis of breast cancer cells than radiotherapy alone (7). In human, however, CR requires high compliance challenges to be maintained for adequate therapeutic period. For these reasons, short period of fasting without malnutrition have been proposed as potentially safe interventions to be associated with cancer treatments (8).

Fasting is commonly defined as a time-controlled deprivation of all kinds of foods and dietary nutrients. Differently to nocturnal fasting, time-controlled fasting leads to a profound metabolic reprogramming building up adaptive stress responses that are involved in life and health span

extension (9–13). However, the adaptive stress responses induced by fasting occurring in normal cells differ from those activated by cancer cells because oncogenes might limit the activation of nutrient-sensing pathways while increasing chemotherapy vulnerability (8). Notably, proto-oncogenes such as IGF1R, PI3K, and AKT activate growth signaling and addict cancer cells to nutrient such as glucose and amino acids to meet their high proliferative rate (8). It has been shown that different cycles of fasting are effective in limiting tumor progression in several murine cancer models (14–17). However, the greatest effects were observed when fasting was combined with the conventional chemotherapy or radiotherapy (14–18). Interestingly, in these studies, fasting interventions alone do not cause clear signs of discomfort, but rather improve the animal condition. When fasting was combined with conventional therapies (e.g., temozolomide), most of the mice appeared healthy with the tumor-size below the controls, indicating that the combination of both treatments is well tolerated and improve tumor-bearing survival (14). The protective role of fasting against the side effects of anticancer therapy was confirmed in another study in which fasting was able to improve the overall cardiac response (maintenance of diastolic/systolic volumes and left ventricle wall thickness) to high-dose of doxorubicin (19). Fasting also exerted a significant protection against reduced mobility, ruffled hair, and hunched back posture caused by high dose of etoposide in mice (20). The anticancer effects of fasting might also rely on ketone bodies increase (21, 22). In support of this assumption, meta-analysis on ketogenic diets (KD), low in carbohydrates and high in fats, suggested a salutary impact on survival in animal models, with benefits prospectively linked to the magnitude of ketosis, time of diet initiation, and tumor location (23). Other evidence also demonstrated that KD might be safely used as adjuvant therapies to conventional radiation and chemotherapies (24). In particular, KD together with conventional radiotherapy led to increased radiation sensitivity in pancreatic cancer xenografts in mice (25). Similar results were obtained in mice bearing lung cancer xenografts (26). However, patients have demonstrated difficulty to comply with a KD while receiving concurrent radiation and chemotherapy in advanced lung and pancreatic cancer (25). Therefore, as better tolerated with respect to CR and KD, fasting appears to be more promising as adjuvant treatment in cancer therapy. Finally, it has been demonstrated that fasting could be replaced by the administration of CR mimetics, which showed the capability to improve the efficacy of chemotherapy as well. However, the objective response rates with metformin (27–30) or rapalogs (31) in clinical trials are still unclear and comparative analyses delineating a selective effectiveness of these drugs in cancer treatment and patient tolerability have to be more deeply elucidated.

NUTRIENT MODULATION IN PROLIFERATING/RESILIENT CANCER CELLS: A MOLECULAR VIEW

The reduced levels of nutrients and growth factors observed during fasting led to hypothesize their mandatory role in governing the differential stress responses in normal and cancer cells (10, 14, 16, 18). The different responses of normal and cancer cells to fasting shed light on their different sensitivity to nutrients and growth factors (18).

IGF-1/IGF-1R signaling is strongly dependent on nutrient availability and involves intensification of cancer cell proliferation, through the direct effects on PI3K/Akt signaling, and resistance to cell death imposed by chemotherapeutics and radiotherapy (**Figure 1**) (32). Indeed, fasting reduces circulating IGF-1 levels and this event protects mice deficient in the liver production of IGF-1 against chemotherapy drugs (16). Accordingly, restoration of IGF-1 was sufficient to reverse the protective effect of fasting (16). Reducing IGF-1 protects primary glia, but not glioma cells, against cyclophosphamide and mouse embryonic fibroblasts against doxorubicin (16). In the opposite manner, IGF-1 supplementation in starved breast cancer cells reversed drug sensitization. Overall, these findings strongly indicate that the fasting-mediated sensitization of cancer cells to chemotherapeutic drugs is conferred by the decrease of IGF-1 levels (15).

Nutrient shortage *per se* is able to increase mitochondrial reactive oxygen species (ROS) production in cancer cells arguing that limiting nutrient availability could enhance the effectiveness of redox-based cancer therapeutics (**Figure 1**) (33, 34). Actually, in breast cancer and melanoma cells, nutrient starvation was found to increase superoxide levels and aggravate oxidative stress caused by cyclophosphamide and cisplatin (15, 35). When applied in combination, fasting and chemotherapy act in synergy in elevating ROS levels and triggering DNA damage also in *in vivo* models of cancer (36). Micro-PET analyses in murine models of colon cancer cells revealed that fasting is effective as oxaliplatin (OXP) in reducing the average tumor glucose consumption and the lowest values were achieved by coupling fasting with OXP. In colon cancer cells, nutrient starvation upregulates oxidative phosphorylation with a significant production in mitochondrial superoxide caused by electron leakage. Consequently, starvation or OXP alone markedly increased ROS generation and their combination (starvation plus OXP) exacerbated ROS production in colon cancer cells (36). The hypothesis that cytotoxicity induced by glucose deprivation in cancer cells is mediated by mitochondrial superoxide and H_2O_2 was confirmed by exposing glucose-deprived transformed human fibroblasts to electron transport chain blockers (ETCBs), known to increase mitochondrial superoxide and H_2O_2 production (37). Glucose deprivation in the presence of ETCBs enhanced oxidative stress as well as cell death in several different human cancer cell lines (PC-3, DU145, MDA-MB231, and HT-29). In addition, human osteosarcoma cells lacking functional mitochondrial electron transport chain [rho(0)] were resistant to glucose deprivation-induced cytotoxicity and oxidative stress in the presence of antimycin A (complex III inhibitor), thus highlighting the role of mitochondrial ROS as mediators of cancer cell death (37).

The mechanisms by which KDs act as adjuvants in cancer therapy also seem to be associated with increased oxidative stress within cancer cells (24). Indeed, upon KD, the high level of circulating fatty acids limits the availability of glucose for glycolysis (Randle's Cycle) (38). This reduces the formation of pyruvate and glucose-6-phosphate and in turn the synthesis of

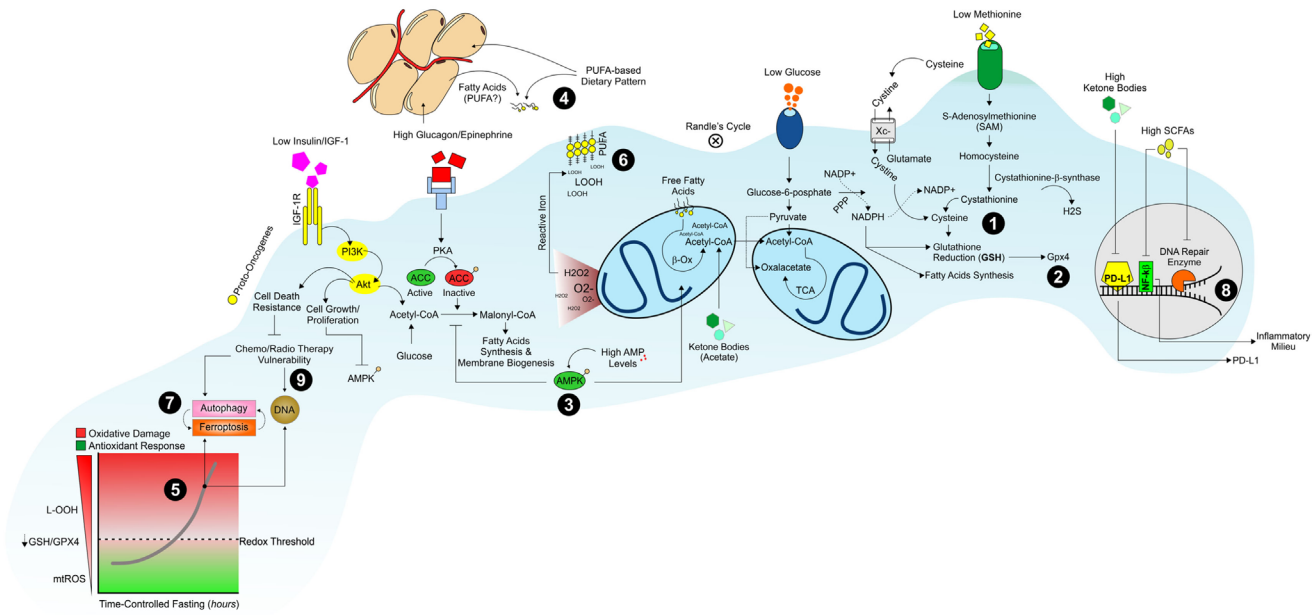

FIGURE 1 | Dietary strategy to promote a hostile metabolism in proliferating/resilient cancer cells. Dietary patterns low in proteins, starch, and sugars promote an environment poor in sulfur amino acids (i.e., methionine, cysteine), glucose, and growth factors (insulin/IGF-1) that could limit NADPH/GSH production (1) and GPX4 activity (2). The diminished levels of glucose and glucagon/insulin ratio switch-off lipid synthesis and switch-on AMPK-driven lipid oxidation pathways in proliferating cancer cells (3). Under such metabolic conditions, cancer cells build-up their membranes by using extracellular dietary-derived and/or white adipose tissues-released fatty acids (4). The concomitant activation of OxPHOS metabolism and reduction in GSH levels are causative of oxidative stress (5) culminating in a massive lipid peroxidation (LOOH) (6) and ferroptosis in cancer cells (7). Diet low in starch, sugars, and proteins but rich in fatty acids also increases ketone bodies and modulates gut microbiota features by producing short-chain fatty acids (SCFAs). Ketone bodies and SCFAs affect PD-L1, nuclear factor-kb, and DNA repair enzymes genes transcription (8) promoting the chemo/radiotherapy vulnerability of cancer cells (9).

NADPH through the pentose phosphate pathway (PPP) (39). NADPH is necessary for buffering hydroperoxides (LOOH) production *via* the NADPH-dependent glutathione/glutathione peroxidase (GSH/GPX) system (40, 41). As consequence, an increase of LOOH is likely elicited (24) (**Figure 1**). Accordingly, hyperketotic diabetic patients have a higher level of lipid peroxidation in erythrocytes membrane and a significant decrease in cellular GSH levels than normal ketonic diabetic patients (42). Treatments with the ketone body acetoacetate elevated the levels of lipid peroxidation in human endothelial cells inhibiting their proliferation (42). This evidence suggests a direct role of ketone bodies in directly affecting GSH levels.

The main non-enzymatic cellular antioxidant GSH acts as an electron donor to reduce oxidized macromolecules, becoming itself oxidized in the process. Oxidized glutathione (GSSG) may then be restored in GSH through the action of the NADPH-dependent glutathione reductase (43). This enzymatic process generates $NADP^+$, which may be reconverted to NADPH using electrons obtained from different biochemical pathways (44). Thus, proliferating cancer cells develop a peculiar metabolic flexibility to maintain a functional redox threshold by regulating NADPH levels through glycolytic flux modulation (33). Indeed, glucose-addicted human cancer cells cultured in a low-glucose medium without serum and amino acids are able to reprogram their metabolism by shifting toward PPP, which sustains the production of NADPH to dampen oxidative stress (33). However, during the initial stages of solid tumor development, when cells migrate to the lumen of lymphatic or blood vessels by loss of attachment (LOA) to the extracellular matrix, the glucose availability could not be sufficient to produce an adequate amount of NADPH and proliferation is inhibited (45). Upon such environmental stress, cancer cells induce adaptive responses consisting in the activation of AMPK signaling that inhibits fatty acid synthesis and triggers fatty acids oxidation to maintain energy production and NADPH levels (46, 47). Although cancer cells build up such adaptive responses, it has been observed that during LOA, cancer cells undergo ATP and NADPH drop and increase ROS production (48). Several papers demonstrated that cancer cells experiencing glucose shortage might maintain their proliferative capacity and membrane biogenesis by the uptake of extracellular lipids (49). Accordingly, extracellular saturated fatty acids supplementation supports the proliferative demand for biomass synthesis of proliferating cells (50, 51). Otherwise, supplementation with polyunsaturated fatty acids (PUFA) induced a significant cytotoxic effect on cancer cells either alone (52–54) or in combination with conventional anticancer therapies (55, 56). Differently to saturated fatty acids, PUFA are strongly susceptible to peroxidation (lipid peroxidation) in *in vivo* systems (57, 58). This appears to be a key mechanism triggering cancer cell death (59). With all this in mind, forcing the changes in the membrane lipids composition by dietary/nutrient enrichment in PUFA might promote an

intrinsic sensitivity toward lipid peroxidation (57, 58, 60) and cancer cell death (**Figure 1**).

NUTRIENT-MEDIATED COMMITMENT TO FERROPTOSIS IN CANCER CELLS

By preserving NADPH levels, cancer cells sustain GPX/GSH activity during nutrient limitation, and this may confer resistance to redox-based chemotherapeutics (61–63). Indeed, many rebel cancer cells use a common trick to evade annihilation; they enter into what is known as a mesenchymal state that is "epithelial-to-mesenchymal" transition, which provides cancer cell resistance to conventional therapeutic regimens (64). It has been demonstrated that high therapy-resistant mesenchymal cancer cells strictly rely on the selenium-dependent GPX4 for survival (65). By using the reducing power of GSH, GPX4 converts potentially toxic L-OOH to non-toxic lipid alcohols (L-OH) (**Figure 1**) (66–68). Accordingly, inactivation of GPX4 through GSH depletion with erastin, or with a direct GPX4 inhibitor, ultimately results in lipid peroxidation in cancer cells (69). It is thus provocative to hypothesize that the evolutionary pressure to maintain the selenium protein GPX4 might correlate with an organism's requirement for an increased PUFA content, which, in turn, renders complex biological activities possible (70).

Uncontrolled lipid peroxidation is causative of the onset of a metabolically regulated cell death called "ferroptosis," which is characterized by the iron-dependent formation of LOOH leading to cell death (**Figure 1**) (71). Sulfur amino acids play a key role in ferroptosis. In particular, agents that inhibit cystine uptake *via* the cystine/glutamate antiporter (XC system), such as sulfasalazine or erastin, arrest tumor growth and induce ferroptosis (72, 73). The uptake of cystine is followed by its NADPH-dependent conversion in cysteine, the rate-limiting amino acid precursor for the GSH biosynthesis (74). Direct depletion of cystine from plasma using an engineered cystine-degrading enzyme conjugate arrests tumor growth and triggers cell death (75). Agents that conjugate to GSH, as well as chemical or genetic inhibition of GSH biosynthesis, disrupt tumor cell growth and induce a ferroptosis-like form of cell death (76). Ferroptosis appears to be an effective cell death mechanism in cancer cells, since lipophilic antioxidant α-tocopherol or iron chelators, such as deferoxamine, efficiently dampen it (77). Hence, the presence of extracellular cysteine and cystine are crucial for growth and proliferation of various types of cancer, as these amino acids maintain GSH levels and prevent oxidative stress (**Figure 1**) (78–80). Because cysteine is limiting in the biosynthesis of GSH, some cancer cells, under cysteine unavailability, make use of the transsulfuration pathway to biosynthesize cysteine from methionine (Met), a dietary essential sulfur amino acid (81, 82). The essentiality of Met in cancer is supported by the evidence that some cancer cells display a higher sensitivity to Met shortage with respect to normal cells (83–87). The first steps of the transsulfuration pathway are the conversion to *S*-adenosylmethionine (SAM) and transfer of the methyl group of SAM to a large variety of methyl acceptors with formation of *S*-adenosylhomocysteine (SAH) (88), which can be then converted to homocysteine (Hcy) by SAH hydrolase (AHCY) (89). Alternatively, Hcy is converted to cystathionine by cystathionine β-synthase (CBS). CBS catalyzes the condensation of Hcy and serine, thereby forming cystathionine, which is subsequently cleaved to cysteine. Furthermore, exogenous cysteine is also essential for several cancer types (glioma, prostate, and pancreatic), as blocking its uptake through the cystine/glutamate antiporter reduces viability due to the cell death caused by uncontrolled oxidative stress (90–92). Similarly, CBS blockage reduces cancer cell proliferation and attenuates growth of patient-derived colon cancer xenografts models (93). Although these findings suggest that fasting or selective nutrient modulation could trigger ferroptotic cell death in cancer cells, a clear evidence linking nutrient availability to ferroptosis is still lacking. Several works demonstrated that starved cancer cells (mainly in amino acids) as well as cells lacking the enzyme producing NADPH from glucose (glucose-6-phosphate dehydrogenase) experience massive ROS production and autophagy-dependent cell death (33, 94, 95). Autophagy is a process described as intracellular removal of damaged organelles by self-degradative process (96). Interestingly, a tight relationship between autophagic cell death and ferroptosis is emerging (97–99). Indeed, it seems that autophagy activation leads to a degradation of ferritin (ferritinophagy) (97), thus increasing the intracellular free iron levels promoting ROS production and ferroptosis (**Figure 1**) (99).

DIETARY STRATEGIES TO BOOST THE IMMUNOMETABOLIC RESPONSES IN CANCER THERAPY

Short-term fasting has a beneficial impact on cancer immunosurveillance (100). In particular, Pietrocola and co-workers demonstrated that fasting or CR-mimicking drugs, induce the depletion of regulatory T cells (which dampen anticancer immunity), thus igniting autophagic flux in murine models of KRAS-induced lung cancers. Accordingly, the inhibitory effect of fasting on tumor growth is lost in cancers that have been rendered autophagy deficient (100). Recently, also, isocaloric diet with protein restriction has been demonstrated to induce an IRE1α-dependent UPR in cancer cells, enhancing cytotoxic $CD8^+$ T cell (a type of effector T lymphocyte)-mediated response against tumors (101).

Similarly to what observed with prolonged fasting (102), cycles of a fasting-mimicking diet (FMD) are effective in increasing hematopoietic cells proliferation and promoting immune system regeneration and modulation (103). Importantly, FMD has stimulatory effect on common lymphoid progenitor cells and $CD8^+$ T cell-dependent cytotoxicity on breast cancer and melanoma cells (**Figure 2**) (17, 102). The presence of cytotoxic $CD8^+$ T cells in the tumor environment [tumor infiltrating lymphocytes (TIL)] is considered a positive outcome of the cancer treatment (104, 105).

$CD8^+$ T cells are influenced by nutrients and other supportive signals that are generally available in their environment. Generally, tumor cells inactivate $CD8^+$ T cells. The suppression of oxidative phosphorylation and an upregulated glycolytic flux

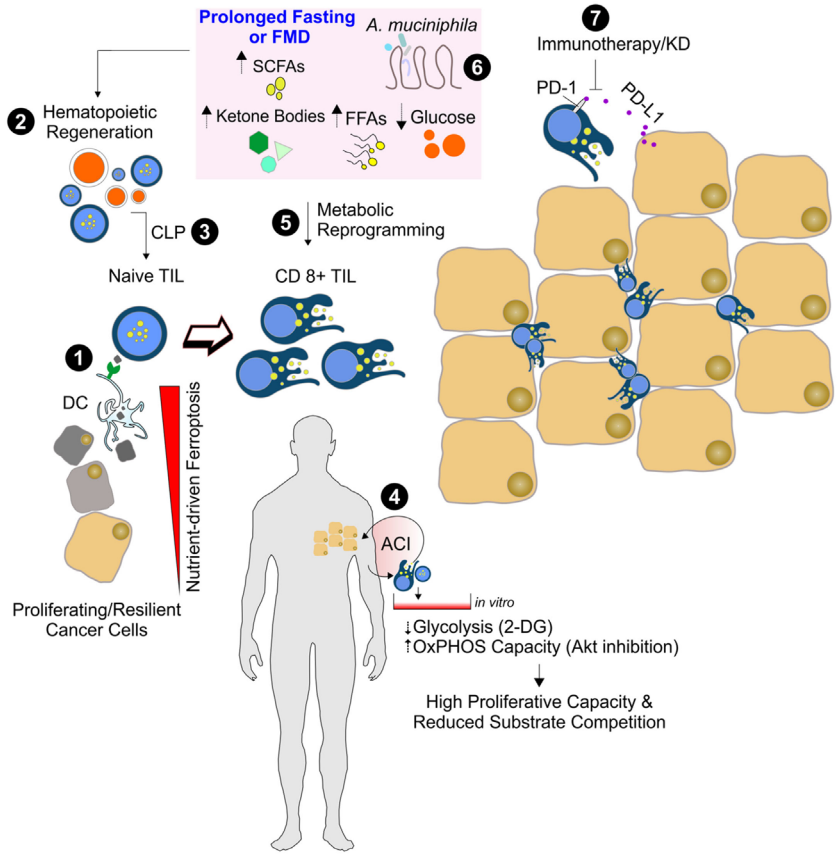

FIGURE 2 | Nutrient manipulation to boost immunometabolic phenotype of CD8⁺ tumor infiltrating lymphocytes (TILs). Naïve CD8⁺ T cells recognize the antigen of ferroptotic cancer cells on class I MHC on dendritic cells, thus becoming mature cytotoxic CD8⁺ T cells (1). After prolonged fasting or fasting-mimicking diet (FMD), an enhanced hematopoietic regeneration rate (2) and enrichment of common lymphoid progenitor cells (CLP) can occur (3). The *in vitro* adoptive T cells immunotherapy (ACI) (4) and *in vivo* nutrient changes (5) reset CD8⁺ TIL metabolism toward mitochondrial oxidative pathways, thus limiting substrate competition with cancer cells and enhancing CD8⁺ TIL-mediated immunosurveillance. Dietary strategies promoting functional gut microbiota changes (e.g., *Akkermansia muciniphila* enrichment) (6) might improve the immune-checkpoint inhibitors (anti PD1/PD-L1) efficacy (7).

of proliferating cancer cells create an immunosuppressive microenvironment (106). Indeed, the glucose-dependent CD8⁺ TIL could undergo a competitive disadvantage for nutrients, and this would negatively affect their immune function. The immunosuppressive metabolic environment could be further enhanced by tumor expression of inhibitory ligands for programmed death 1 receptor (PD-1) which, when bound to their cognate receptors on T cells, limits T cell-intrinsic glucose uptake and glycolysis (107, 108). It has been reported that KD significantly reduces the expression of the inhibitory ligand PD-1 (PD-L1) on CD8⁺ TIL (109). Additionally, mice fed with KD have reduced expression of PD-L1 on the cancer cells that notoriously inhibits CD8⁺ T cells activity (109). This suggests that KD may alter tumor-mediated T cell suppression by reducing the number of cells that are susceptible to inhibition through the PD-1 inhibitory pathway (**Figure 2**).

Nowadays, there has been intense interest in developing adoptive T cells immunotherapy (ACI), which consists in reintroducing into a patient T cells that are previously activated and expanded *in vitro* (110, 111). The success of the ACI depends on the replicative capacity of implanted T cells. A large amount of research has been directed in optimizing T cell activation and using appropriate adjuvants for ACI. However, few experimental studies have been focused on manipulating metabolic pathways that could potentially enhance immunotherapy efficacy. When posed in culture, T cells dispose of a high glucose availability, which is far from the glucose physiological levels especially in the tumor environment (112, 113). Thus, once reintroduced in patients, T cells suffer from low glucose levels and show a moderate survival and replicative capacity. It has been reported that limiting glycolysis in cultured T cells can increase their longevity without inhibiting proliferative capacity (114, 115) (**Figure 2**). Another potential way to enhance the replicative capacity and longevity of ACI cells is promoting oxidative phosphorylation and mitochondrial biogenesis *via* the inhibition of glucose-related signaling pathway that ultimately leads to *in vivo* persistence and improved antitumor immunity (116). The metabolic reprogramming of infiltrating glycolytic lymphocytes toward a catabolic state reliant on fatty acid oxidation appears to assure the success of immunotherapy (113). In line with this assumption, it was

recently demonstrated that the enhancement of lipid catabolism in CD8+ T cells increases the efficacy of immunotherapy within a tumor microenvironment low in glucose (117). In a mouse model of malignant glioma, an enhanced cytolysis *via* tumor-reactive CD8+ T cells was also achieved by ketogenic diet (109). The immunometabolic reprogramming necessary for CD8+ TIL could at least partially explain the mechanism by which KD or fasting enhances cytotoxic effect against cancer cells. Such diets are indeed powerful in inducing a cellular metabolic shift from glycolysis toward FAO.

It is now emerging that CD8+ TIL response to immune checkpoint blockade inhibitor PD1 can be also modulated by gut microbiota (118–120). A very recent paper has revealed that fecal microbiota from patients affected with metastatic melanoma and responsive to anti-PD1 therapy display increased abundance of *Akkermansia muciniphila*. *A. muciniphila* introduction into mice receiving human nonresponder fecal microbiota transplant improved antitumor immune CD8+ T cell infiltration and activity and increased anti-PD1 therapy efficacy (120, 121). Another intriguing observation is that *Faecalibacterium* and *Bifidobacterium* are associated with anti-inflammatory responses, a regulatory arm of the immune system that aims to prevent over-activation of the immune response and restores host homeostasis (120). Given that changes in host metabolism and microbiota can occur in tandem, it was hypothesized that gut microbial diversity and composition are predictors of the response to

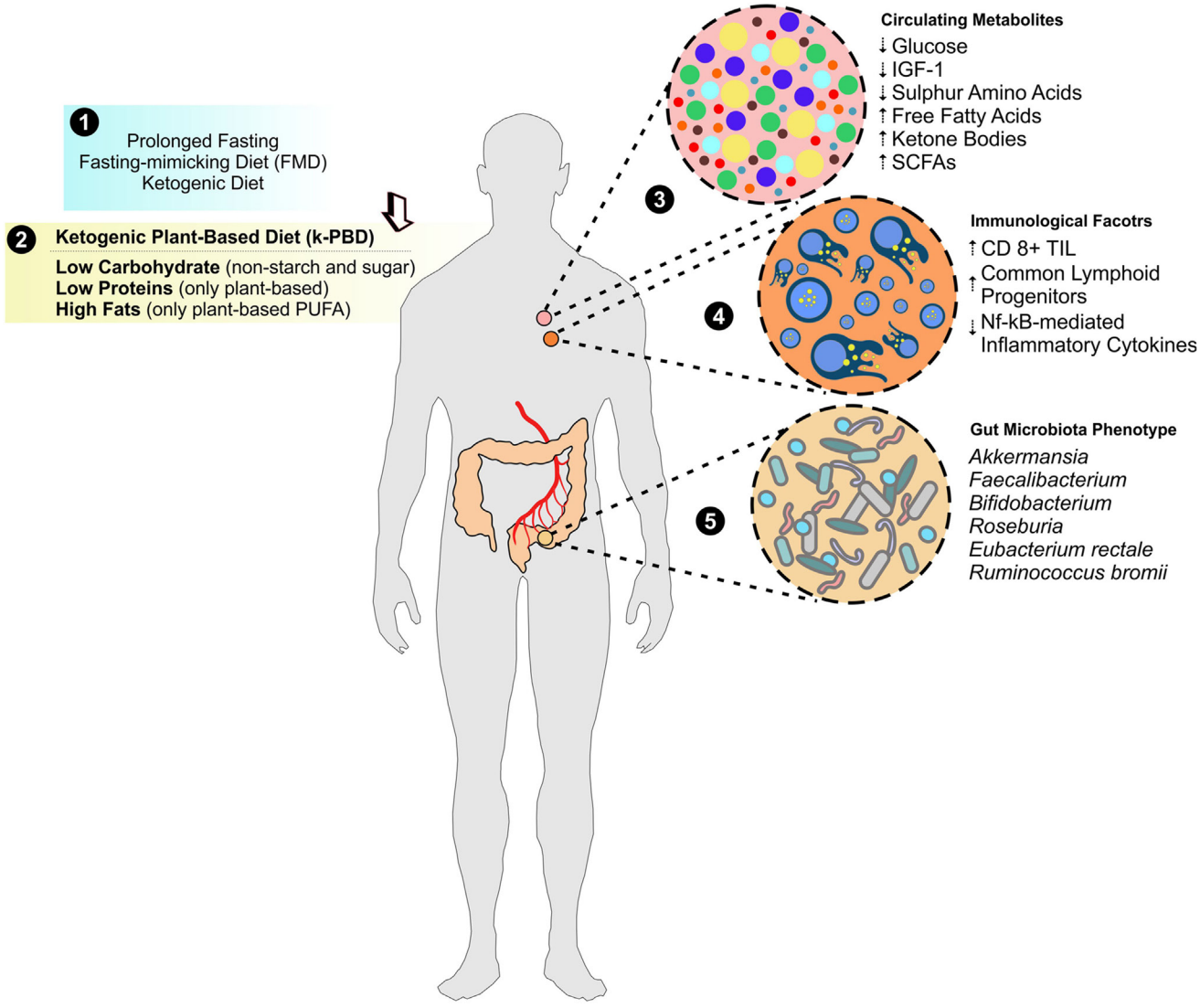

FIGURE 3 | Improving metabolites and immunological anticancer profile by k-PBD. Evidence from prolonged fasting, fasting-mimicking diet (FMD), and ketogenic diet demonstrated a strong usefulness as adjuvants in cancer therapy (1). In this issue, we propose a moderate ketogenic plant-based diet (k-PBD), low in carbohydrates (starch and sugars in particular) and animal proteins (poor in sulfur amino acids and selenium) but rich in fats [mainly in vegetable polyunsaturated fatty acids (PUFA)] (2), which could strongly modulate circulating metabolites (3), immunological factors (4), and gut microbiota asset (5) that overall create a hostile environment to cancer cells.

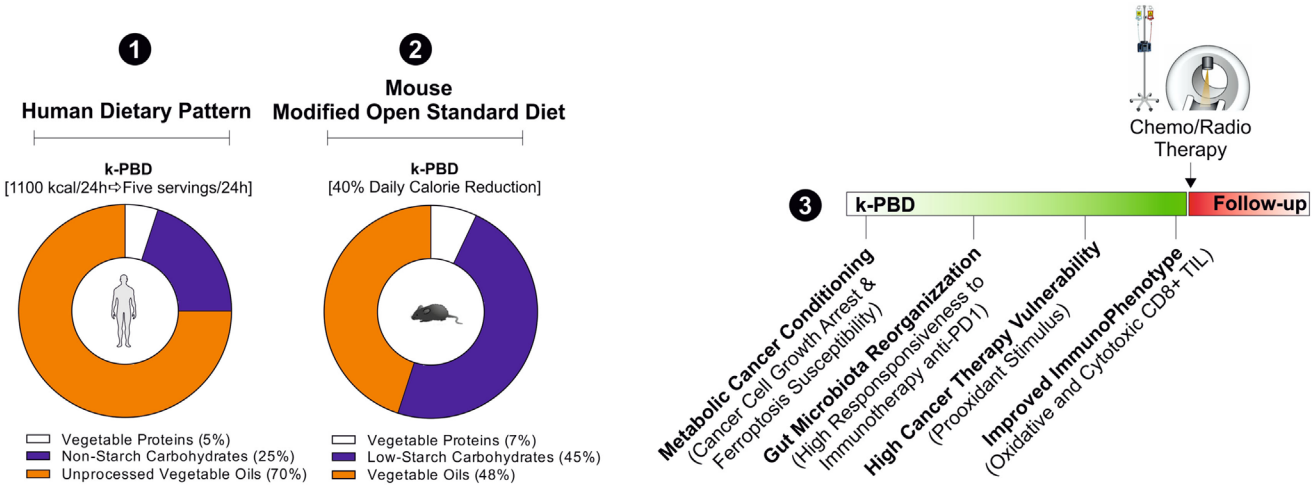

FIGURE 4 | Hypothetical cancer phenotypic responses to k-PBD. For the human dietary intervention studies, k-PBD (for 4 consecutive days; 5 servings each day) should provide about 1,100 kcal/die with 5% of calories from vegetable proteins, 25% from carbohydrates (non-starchy vegetables), and 70% from unprocessed vegetable oils (1). k-PBD proposed for murine cancer models should be reduced in total calories (40% reduction versus *ad libitum* diet), provide 7% calories from vegetable proteins, 45% from low starch carbohydrates, 48% in vegetable oils (2). The proposed dietary pattern should be started prior to conventional cancer therapies (3).

cancer therapy (121) (**Figure 2**). Accordingly, germ-free mice implanted with human tumor cells and transplanted with feces from chemotherapy responders showed an ameliorated response to chemotherapy than mice colonized with microbiota from nonresponder patients (119).

The diet has a strong capacity to rapidly and reproducibly reshape the gut microbiome (122). Indeed, fasting or plant-based diet remodels microbial community structure and overwhelms interindividual differences in microbial gene expression. The animal-based diets are known to increase the abundance of bile-tolerant microorganisms (*Alistipes*, *Bilophila*, and *Bacteroides*) and decrease the levels of the high fermentative *Firmicutes* that metabolize dietary plant polysaccharides (*Roseburia, Eubacterium rectale*, and *Ruminococcus bromii*) (122). More recently, it has been demonstrated that alternate day fasting shifts the gut microbiota composition from *Bacteroides* to *Firmicutes* leading to elevation of the fermentation products (123). Plant-based foods are mainly characterized by resistant starches and dietary fibers and promote their gut microbiota-mediated fermentation and decomposition. These processes provide additional amount of short chain fatty acids (SCFAs) to the host (124) (**Figure 2**). The major SCFAs, i.e., acetate, propionate, and butyrate, have different production ratios and physiological activities. Through ^{1}H NMR-based metabolomics, it was revealed that mice treated with alternate day fasting increased acetate levels both in the cecum and sera (123). Acetate, when ligated to coenzyme A (acetyl-CoA), is among the most central and dynamic metabolites in intermediary metabolism. Under stressful circumstance (e.g., fasting-like conditions), cancer cells may convert extracellular acetate to acetyl-CoA, thus promoting the biogenesis of membrane building blocks that sustain the high proliferative rate. This adaptive response involves the cytosolic form of short-chain acyl-CoA synthetases (ACC2). Accordingly, increased ACC2 protein levels were detected in a subset of human triple negative breast cancer samples, and such an elevation correlates with poor survival (125). Differently to acetate, butyrate shows many regulatory properties including the inhibition of histone deacetylases. Histone deacetylase inhibitors (HDACi's) are emerging as promising anticancer drugs when administered alone or in combination with chemotherapeutic agents or radiotherapy. Previous research suggests that HDACi's have a high degree of selectivity for killing cancer cells. For instance, the HDACi sodium butyrate suppresses DNA double strand break repair induced by etoposide more efficiently in MCF-7 cells than in HEK293 cells (126). Sodium butyrate alone also resulted in accumulation of ROS, DNA double-strand breaks, and apoptosis in HCT-116 colon cancer cell lines; when combined with mitomycin C or radiotherapy, sodium butyrate increases sensitivity of cancer cells to the drug (127, 128). In animal models of gastric carcinoma, sodium butyrate was found to inhibit tumor mass formation and increase tumor infiltration by $CD8^{+}$ TIL (129). Finally, several studies also demonstrated a strong effectiveness of SCFA to inactivate nuclear factor-kb by downregulating the production of the pro-inflammatory cytokine TNFα (130–134), which is commonly activated to promote a pro-carcinogenic environmental milieu (135) (**Figure 1**).

CONCLUSION AND PERSPECTIVE

Despite recent advances have been made in cancer therapy, the prognosis for many cancer patients remains poor, and current treatments still show severe adverse events. Thus, finding complementary treatments that have limited patient toxicity and simultaneously enhance therapy responses in cancer versus normal cells is urgent. Diet has a strong capacity to modulate cell responses to environmental stimuli and shows great potential in

improving cancer prognosis. The mechanisms by which dietary nutrients enhance anticancer effects of standard anticancer therapies (chemotherapy, radiotherapy, immunotherapy) has not been fully elucidated yet. Preclinical studies have demonstrated the safety and efficacy of specific dietary interventions in counteracting tumor progression during anticancer therapy in murine models. However, most of the data present in the literature take advantage of the use of mice and this may limit the translation to clinical research. Therefore, a huge amount of work is now necessary to confirm these very promising results in humans.

Deprivation of nutrients (e.g., glucose, sulfur amino acids) as well as of nutrient-responsive growth factors (e.g., IGF-1) seems to selectively kill high proliferative/resilient cancer cells by forcing their glycolytic asset toward an oxidative metabolism (i.e., fatty acids and ketone bodies as energy sources) and limiting GPX activity as consequence of reduced GSH levels. Nutrient scarcity also improves immunometabolism enhancing cytotoxic efficiency of $CD8^+$ TIL within the tumor mass through, probably, the concomitant gut microbiota and immunometabolic rearrangements (**Figure 3**).

Herein, we propose weekly cycles of 4 days of a plant-based moderate ketogenic diet (k-PBD) that could reprogram systemic metabolism conferring a hostile environment to cancer cells (**Figure 3**). In particular, k-PBD should be low in proteins (mainly vegetable proteins low in sulfur amino acids and selenium), carbohydrates (non-starchy vegetables), and high in lipids (mainly unprocessed vegetable oils rich in PUFA). Remarkably, even though not supported by experimental data, it is highly expected that this diet could be able to increase ketonemia as it contains high amounts of fats concomitantly to reduced calories. This diet could increase the efficiency of $CD8^+$ TIL, by reprogramming their metabolism (fat-dependent metabolism) to better counteract the metabolic features of proliferating cancer cells (glucose-dependent metabolism) and sensitize cancer cells to the therapy. The k-PBD could be consumed prior to conventional cancer therapies (e.g., prior each cycle of chemotherapy or prior a single fraction of radiation therapy). With this composition and time of treatment, k-PBD could be effective in: (i) changing the membrane chemistry by PUFA enrichment (high peroxidation index); (ii) reducing the sulfur-dependent antioxidant power (lowering NADPH, GSH, GPX4); (iii) forcing the metabolic shift toward mitochondrial metabolism in cancer cells. Furthermore, the high fermentative fibers of k-PBD could induce a functional microbiota reshaping improving immunotherapy efficacy (e.g., anti-PD1 therapy) (**Figure 4**).

AUTHOR CONTRIBUTIONS

DL-B conceptualized and wrote the manuscript. KA performed critical revision of the manuscript for intellectual content.

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Association of Frequency of Organic Food Consumption With Cancer Risk

Findings From the NutriNet-Santé Prospective Cohort Study

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IMPORTANCE Although organic foods are less likely to contain pesticide residues than conventional foods, few studies have examined the association of organic food consumption with cancer risk.

OBJECTIVE To prospectively investigate the association between organic food consumption and the risk of cancer in a large cohort of French adults.

DESIGN, SETTING, AND PARTICIPANTS In this population-based prospective cohort study among French adult volunteers, data were included from participants with available information on organic food consumption frequency and dietary intake. For 16 products, participants reported their consumption frequency of labeled organic foods (never, occasionally, or most of the time). An organic food score was then computed (range, 0-32 points). The follow-up dates were May 10, 2009, to November 30, 2016.

MAIN OUTCOMES AND MEASURES This study estimated the risk of cancer in association with the organic food score (modeled as quartiles) using Cox proportional hazards regression models adjusted for potential cancer risk factors.

RESULTS Among 68 946 participants (78.0% female; mean [SD] age at baseline, 44.2 [14.5] years), 1340 first incident cancer cases were identified during follow-up, with the most prevalent being 459 breast cancers, 180 prostate cancers, 135 skin cancers, 99 colorectal cancers, 47 non-Hodgkin lymphomas, and 15 other lymphomas. High organic food scores were inversely associated with the overall risk of cancer (hazard ratio for quartile 4 vs quartile 1, 0.75; 95% CI, 0.63-0.88; *P* for trend = .001; absolute risk reduction, 0.6%; hazard ratio for a 5-point increase, 0.92; 95% CI, 0.88-0.96).

CONCLUSIONS AND RELEVANCE A higher frequency of organic food consumption was associated with a reduced risk of cancer. Although the study findings need to be confirmed, promoting organic food consumption in the general population could be a promising preventive strategy against cancer.

+ Invited Commentary
+ Author Audio Interview
+ Supplemental content

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Worldwide, the number of new cases of cancer was estimated in 2012 at more than 14 million,[1,2] and cancer remains one of the leading causes of mortality in France. Among the environmental risk factors for cancer, there are concerns about exposure to different classes of pesticides, notably through occupational exposure.[3] A recent review[4] concluded that the role of pesticides for the risk of cancer could not be doubted given the growing body of evidence linking cancer development to pesticide exposure. While dose responses of such molecules or possible cocktail effects are not well known, an increase in toxic effects has been suggested even at low concentrations of pesticide mixtures.[5]

Meanwhile, the organic food market continues to grow rapidly in European countries,[6] propelled by environmental and health concerns.[7-10] Organic food standards do not allow the use of synthetic fertilizers, pesticides, and genetically modified organisms and restrict the use of veterinary medications.[11] As a result, organic products are less likely to contain pesticide residues than conventional foods.[12,13] According to a 2018 European Food Safety Authority[13] report, 44% of conventionally produced food samples contained 1 or more quantifiable residues, while 6.5% of organic samples contained measurable pesticide residues. In line with this report, diets mainly consisting of organic foods were linked to lower urinary pesticide levels compared with "conventional diets" in an observational study[14] of adults carried out in the United States (the median dialkyphosphate concentration among low organic food consumers was 163 nmol/g of creatinine, while among regular organic food consumers it was reduced to 106 nmol/g of creatinine). This finding was more marked in a clinical study[15] from Australia and New Zealand (a 90% reduction in total dialkyphosphate urinary biomarkers was observed after an organic diet intervention) conducted in adults.

Because of their lower exposure to pesticide residues, it can be hypothesized that high organic food consumers may have a lower risk of developing cancer. Furthermore, natural pesticides allowed in organic farming in the European Union[16] exhibit much lower toxic effects than the synthetic pesticides used in conventional farming.[17] Nevertheless, only 1 study[18] to date has focused on the association between frequency of organic food consumption and cancer risk, reporting a lower risk of non-Hodgkin lymphoma (NHL) only. However, consumption of organic food was assessed using only a basic question. Multiple studies[19-24] have reported a strong positive association between regular organic food consumption and healthy dietary habits and other lifestyles. Hence, these factors should be carefully accounted for in etiological studies in this research field. In the present population-based cohort study among French adult volunteers, we sought to prospectively examine the association between consumption frequency of organic foods, assessed through a score evaluating the consumption frequency of organic food categories, and cancer risk in the ongoing, large-scale French NutriNet-Santé cohort. The follow-up dates of the study were May 10, 2009, to November 30, 2016.

Key Points

Question What is the association between an organic food–based diet (ie, a diet less likely to contain pesticide residues) and cancer risk?

Findings In a population-based cohort study of 68 946 French adults, a significant reduction in the risk of cancer was observed among high consumers of organic food.

Meaning A higher frequency of organic food consumption was associated with a reduced risk of cancer; if the findings are confirmed, promoting organic food consumption in the general population could be a promising preventive strategy against cancer.

Methods

Study Population

The NutriNet-Santé study is a web-based prospective cohort in France aiming to study the associations between nutrition and health, as well as the determinants of dietary behaviors and nutritional status. This cohort was launched in 2009 and has been previously described in detail.[25] Volunteers with access to the internet are recruited from the general population and complete online self-administrated questionnaires using a dedicated website.

The NutriNet-Santé study is conducted in accord with the tenets of the Declaration of Helsinki.[26] It was approved by the institutional review board of the French Institute for Health and Medical Research and the Commission Nationale de l'Informatique et des Libertés. The study is registered at ClinicalTrials.gov (NCT03335644). Electronic informed consent was obtained from each participant.

Data Collection

The baseline questionnaires investigating sociodemographics and lifestyles, health status, physical activity, anthropometrics, and diet were pilot tested and then compared against traditional assessment methods or objectively validated.[27-32] Two months after enrollment, volunteers were asked to provide information on their consumption frequency of 16 labeled organic products (fruits; vegetables; soy-based products; dairy products; meat and fish; eggs; grains and legumes; bread and cereals; flour; vegetable oils and condiments; ready-to-eat meals; coffee, tea, and herbal tea; wine; biscuits, chocolate, sugar, and marmalade; other foods; and dietary supplements). Consumption frequencies of organic foods were reported using the following 8 modalities: (1) most of the time, (2) occasionally, (3) never ("too expensive"), (4) never ("product not available"), (5) never ("I'm not interested in organic products"), (6) never ("I avoid such products"), (7) never ("for no specific reason"), and (8) "I don't know." For each product, we allocated 2 points for "most of the time" and 1 point for "occasionally" (and 0 otherwise). The 16 components were summed to provide an organic food score (range, 0-32 points).

At study inclusion, dietary intake was assessed using three 24-hour records, randomly allocated over a 2-week period, including 2 weekdays and 1 weekend day, with a validated method.[30] Participants reported all foods and beverages consumed at each eating occasion. Portion sizes were estimated using photographs from a previously validated picture booklet[33] or directly entered as grams, volumes, or purchased units. Alcohol intake was calculated using either the 24-hour records or a frequency questionnaire for those identified as abstainers in the three 24-hour record days. Similarly, the weekly consumption of seafood was assessed by a specific frequency question. Daily mean food consumption was calculated from the three 24-hour records completed at inception and weighted for the type of day (weekday or weekend day). Ultraprocessed food consumption was assessed using the NOVA classification.[34,35]

Nutrients intakes were derived from individuals' food intakes assessed via the 24-hour records and were calculated using the NutriNet-Santé food composition table.[36] Underreporters were identified and excluded using the method by Black.[37]

Diet quality was assessed using a modified version of the validated Programme National Nutrition Santé Guideline Score without the physical activity component (mPNNS-GS), reflecting adherence to the official French nutritional guidelines.[38] Components, cutoffs, and scoring are summarized in eTable 1 in the Supplement.

At baseline, data on age, sex, occupational status, educational level, marital status, monthly income per household unit, number of children, and smoking status were collected. Monthly income per household unit was calculated by dividing the household's total monthly income by the number of consumption units.[39] The following categories of monthly income per household unit were used: less than €1200 (less than US $1377.46), €1200 to €1800 (US $1377.46 to US $2066.18), greater than €1800 to €2700 (greater than US $2066.18 to US $3099.28), and greater than €2700 (greater than US $3099.28). Physical activity was assessed by the International Physical Activity Questionnaire.[40]

Anthropometric questionnaires provided information on weight and height. The use of dietary supplements (yes or no) and sun exposure were assessed using specific questionnaires. For sun exposure, the question was formulated as follows: "During adulthood, have you been regularly exposing yourself to the sun?" (yes or no).

Case Ascertainment

Participants self-declared health events through a yearly health status questionnaire or using an interface on the study website allowing the entering of health events at any time. For each reported cancer case, individuals were asked by a study physician (P.G. and other nonauthors) to provide their medical records (diagnoses, hospitalizations, etc). The study physicians contacted the participants' treating physician or the respective hospitals to collect additional information if necessary. All medical information was collegially reviewed by an independent medical expert committee for the validation of major health events. Overall, medical records were obtained for more than 90% of self-reported cancer cases. Linkage of our data (decree authorization in the Council of State No. 2013-175) to medicoadministrative registers of the national health insurance system (Système National d'Information Inter-Régimes de l'Assurance Maladie [SNIIRAM] databases) allowed complete reporting of health events. Mortality data were also used from the French Centre for Epidemiology Medical Causes of Death database (CépiDC). Cancer cases were classified using the *International Statistical Classification of Diseases and Related Health Problems, 10th Revision, Clinical Modification*.[41] In this study, all first primary cancers diagnosed between study inclusion and November 30, 2016, were considered cases except for basal cell skin carcinoma, which was not considered cancer.

Statistical Analysis

For the present study, we used data from volunteers who were enrolled before December 2016 who completed the organic food questionnaire (n = 95 123) and did not have prevalent cancer (except for basal cell skin carcinoma) (n = 89 711), with a final population of 68 946 adults who had available data for the computation of the mPNNS-GS and follow-up data. The studied sample was compared with participants who were in the eligible population but who were excluded because of missing data (eTable 2 in the Supplement). To date, the dropout rate in the NutriNet-Santé cohort is 6.7%.

Baseline characteristics are presented by quartile (Q) of the organic food score. Cox proportional hazards regression models with age as time scale were used to estimate hazard ratios (HRs) and 95% CIs, reflecting the association between the organic food score (as a continuous variable, while modeling the HR associated with each 5-point increase, and as quartiles, with the first quartile as reference) and the incidence of overall cancer. A 5-point increment corresponded to half of the interquartile range. Tests for linear trend were performed using quartiles of the organic food score as an ordinal variable. Full details about cancer risk modeling are provided in the eAppendix in the Supplement, with additional information included in eTables 3 through 6 in the Supplement.

All statistical tests were 2 sided, and $P < .05$ was considered statistically significant. A statistical software program (SAS, version 9.4; SAS Institute Inc) was used for analyses.

Results

The mean (SD) follow-up time in our study sample was 4.56 (2.08) years; 78.0% of 68 946 participants were female, and the mean (SD) age at baseline was 44.2 (14.5) years. During follow-up, 1340 first incident cancer cases were identified, with the most prevalent being 459 breast cancers (34.3%), 180 prostate cancers (13.4%), 135 skin cancers (melanoma and spinocellular carcinoma) (10.1%), 99 colorectal cancers (7.4%), 47 NHLs (3.5%), and 15 other lymphomas (1.1%).

Baseline Characteristics of the Sample

Higher organic food scores were positively associated with female sex, high occupational status or monthly income per

household unit, postsecondary graduate educational level, physical activity, and former smoking status (**Table 1**). Higher organic food scores were also associated with a higher mPNNS-GS. Dietary characteristics by organic food score quartiles are summarized in eTable 7 in the Supplement. Higher organic food scores were associated with a healthier diet rich in fiber, vegetable proteins, and micronutrients. Higher organic food scores were also associated with higher intake of fruits, vegetables, nuts, and legumes and with lower intake of processed meat, other meat, poultry, and milk.

Organic Food Score in Relation to Cancer Risk

The association between the organic food score and the overall risk of cancer is summarized in **Table 2**. After adjustment for confounders (main model), high organic food scores were linearly and negatively associated with the overall risk of cancer (HR for Q4 vs Q1, 0.75; 95% CI, 0.63-0.88; *P* for trend = .001; absolute risk reduction, 0.6%; HR for a 5-point increase, 0.92; 95% CI, 0.88-0.96). Accounting for other additional dietary factors did not modify the findings. After removing early cases of cancers (eTable 5 in the Supplement), the overall association remained significant (HR for Q4 vs Q1, 0.70; 95% CI, 0.56-0.88; *P* for trend = .004).

Combining both a high-quality diet and a high frequency of organic food consumption did not seem to be associated with a reduced risk of overall cancer compared with a low-quality diet and a low frequency of organic food consumption. Negative associations were found between the risk of cancer and combining both a low- to medium-quality diet and a high frequency of organic food consumption (eTable 6 in the Supplement).

Population attributable risks (PAR) were calculated[42] from multivariable-adjusted HRs (main model) in relation to the organic food score and a family history of cancer to identify how much of the risk was specifically attributable to the organic food score. Herein, PAR represents the proportion of cancer cases that can be attributed to any risk factor studied. By comparison, the number of avoided cancers (all types of cancer) owing to a high organic food consumption frequency was slightly lower than the estimated number of cases owing to a family history of cancer (% PAR high organic food score of −6.78 vs % PAR family history of cancer of 8.93) under the causality assumption.

Associations by cancer site are summarized in **Table 3**. Our findings revealed a negative association between high organic food scores and postmenopausal breast cancer, NHL, and all lymphomas. No associations were observed with other cancer sites.

Sensitivity Analysis

When applying a simplified, plant-derived organic food score, our main findings were not substantially changed except for postmenopausal breast cancer, for which the association with the organic food score did not remain significant (**Table 4**). When stratifying by various factors, significant associations were detected in women, older individuals, those with lower and higher educational levels, individuals with a family history of cancer, those with low to medium overall nutritional quality, all body mass index strata, and former smokers (**Figure**).

Discussion

In this large cohort of French adults, we observed that a higher organic food score, reflecting a higher frequency of organic food consumption, was associated with a decreased risk of developing NHL and postmenopausal breast cancer, while no association was detected for other types of cancer. Epidemiological research investigating the link between organic food consumption and cancer risk is scarce, and, to the best of our knowledge, the present study is the first to evaluate frequency of organic food consumption associated with cancer risk using detailed information on exposure. Therefore, frequency of organic food consumption for various food groups was assessed, and our models were adjusted for multiple important confounding factors (sociodemographics, lifestyles, and dietary patterns). Control for dietary patterns is of high importance because the current state of research in nutritional epidemiology emphasizes the strong associations between Western and healthy dietary patterns and the development of certain types of cancers.[43-45]

Our results contrast somewhat with the findings from the Million Women Study[18] cohort among middle-aged women in the United Kingdom. In that large prospective study carried out among 623 080 women, consumption of organic food was not associated with a reduction in overall cancer incidence, while a small increase in breast cancer incidence was observed among women who reported usually or always eating organic food compared with women who reported never eating organic food. Moreover, despite different populations and assessment methods, similar results in that study and in our study were obtained with respect to NHL (in the Million Women Study, there was a 21% lower risk among regular organic food consumers compared with nonconsumers).

One possible explanation for the negative association observed herein between organic food frequency and cancer risk is that the prohibition of synthetic pesticides in organic farming leads to a lower frequency or an absence of contamination in organic foods compared with conventional foods[46,47] and results in significant reductions in pesticide levels in urine.[48] In 2015, based on experimental and population studies, the International Agency for Research on Cancer[49] recognized the carcinogenicity of certain pesticides (malathion and diazinon were classified as probably carcinogenic for humans [group 2A], and tetrachlorvinphos and parathion were classified as possibly carcinogenic for humans [group 2B]). While there is a growing body of evidence supporting a role of occupational exposure to pesticides for various health outcomes and specifically for cancer development,[4,50,51] there have been few large-scale studies conducted in the general population, for whom diet is the main source of pesticide exposure.[52] It now seems important to evaluate chronic effects of low-dose pesticide residue exposure from the diet and potential cocktail effects at the general population level. In particular, further research is required to identify which specific factors are responsible for potential protective effects of organic food consumption on cancer risk.

Table 1. Baseline Characteristics According to Quartiles of the Organic Food Score, NutriNet-Santé Cohort, France, 2009 to 2016

Characteristic	Q1	Q2	Q3	Q4	*P* Value[a]
Organic food score, mean (SD), range, 0-32 points	0.72 (0.82)	4.95 (1.41)	10.36 (1.69)	19.36 (4.28)	<.001
Participants, No.	16 831	17 644	17 240	17 231	NA
Age, mean (SD), y	42.99 (15.24)	43.31 (14.78)	44.72 (14.30)	45.89 (13.37)	<.001
Female, %	74.2	78.2	78.7	80.9	<.001
Month of inclusion, mean (SD)[b]	6.08 (2.56)	6.11 (2.57)	6.10 (2.74)	5.99 (2.93)	.002
Occupational status, %					
Unemployed	5.9	5.7	5.6	6.3	<.001
Student	9.5	9.3	7.3	4.6	
Self-employed, farmer	1.6	1.7	1.7	2.5	
Employee, manual worker	24.4	20.6	17.4	14.9	
Intermediate professions	16.3	17.6	17.8	18.8	
Managerial staff, intellectual profession	17.8	21.6	25.9	29.1	
Retired	19.0	17.9	18.9	17.8	
Never employed	5.5	5.6	5.4	6.1	
Educational level, %					
Unidentified	0.6	0.7	0.5	0.8	<.001
<High school diploma	22.8	19.4	16.4	14.4	
High school diploma	19.7	17.4	15.7	13.7	
Postsecondary graduate	56.9	62.6	67.4	71.1	
Marital status, %					
Cohabiting	79.6	80.6	81.8	85.3	<.001
Monthly income per household unit, €, %[c]					
<1200	20.7	17.0	14.0	13.2	<.001
1200 to 1800	26.9	25.3	22.9	23.5	
>1800 to 2700	21.2	23.3	24.7	25.6	
>2700	18.8	22.4	27.6	28.2	
Unwilling to answer	12.4	12.0	10.8	9.5	
Physical activity, %[d]					
Low, <30 min of brisk walking per day or equivalent	26.8	27.3	29.5	31.3	.03
Moderate, 30 to <60 min of brisk walking per day or equivalent	33.7	37.3	38.8	40.6	
High, ≥60 min of brisk walking per day or equivalent	24.0	20.8	19.0	17.2	
Missing data	15.5	14.5	12.7	11.0	
Smoking status, %					
Never smoker	52.0	51.9	50.2	49.4	<.001
Former smoker	31.6	31.9	34.5	36.8	
Current smoker	16.4	16.2	15.3	13.8	
Alcohol intake, mean (SD), g/d	8.34 (13.84)	8.18 (13.11)	8.17 (12.19)	7.54 (11.30)	
Family history of cancer, %	33.8	34.5	36.8	38.6	<.001
BMI, mean (SD)	24.46 (4.92)	23.92 (4.63)	23.64 (4.32)	22.92 (3.89)	<.001
Height, mean (SD), cm	166.91 (8.27)	166.58 (8.05)	166.54 (8.11)	166.40 (7.98)	<.001
Energy intake, mean (SD), kcal/d[e]	1881.71 (493.19)	1855.10 (469.03)	1848.42 (474.71)	1841.24 (464.11)	<.001
mPNNS-GS, mean (SD)	7.41 (1.72)	7.70 (1.71)	7.95 (1.71)	8.19 (1.69)	<.001
Fiber intake, mean (SD), g/d	17.88 (6.55)	18.87 (6.84)	20.05 (7.20)	22.60 (8.31)	<.001
Processed meat intake, mean (SD), g/d	23.67 (29.40)	21.15 (27.12)	18.85 (24.92)	15.12 (22.49)	<.001
Red meat intake, mean (SD), g/d	48.72 (44.51)	44.59 (41.44)	40.77 (40.67)	31.44 (36.81)	<.001
Parity, mean (SD)[f]	1.26 (1.26)	1.27 (1.23)	1.34 (1.23)	1.41 (1.21)	<.001
Postmenopausal status, %[f]	16.6	19.3	22.4	24.7	<.001
Use of hormonal treatment for menopause, %[f]	4.0	4.6	5.0	4.9	.01
Use of oral contraception, %[f]	24.7	23.2	19.1	14.0	<.001

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); mPNNS-GS, Programme National Nutrition Santé Guideline Score without the physical activity component; NA, not applicable; Q, quartile.

[a] *P* value based on linear trend for continuous variables or Mantel-Haenszel χ^2 test for categorical variables.

[b] This category indicates the month of the year during which the particpant was included.

[c] In 2018 US dollars, the monetary ranges are "less than $1377.46," "$1377.46 to $2066.18," "greater than $2066.18 to $3099.28," and "greater than $3099.28."

[d] Physical activity levels assessed using the French short form of the International Physical Activity Questionnaire self-administered online.

[e] Energy intake without alcohol.

[f] For women.

Table 2. Multivariable Associations Between the Organic Food Score (Modeled as a Continuous Variable and as Quartiles) and Overall Cancer Risk, NutriNet-Santé Cohort, France, 2009 to 2016

Variable	HR (95% CI)				*P* Value for Trend[a]	HR (95% CI) for a 5-Point Increase	*P* Value
	Q1	Q2	Q3	Q4			
Cases/noncases	360/16471	358/17286	353/16887	269/16962	NA	NA	NA
Model 1[b]	1 [Reference]	0.93 (0.80-1.07)	0.90 (0.78-1.04)	0.70 (0.60-0.83)	<.001	0.91 (0.87-0.94)	<.001
Model 2[c]	1 [Reference]	0.94 (0.81-1.09)	0.92 (0.79-1.07)	0.75 (0.63-0.88)	.001	0.92 (0.88-0.96)	<.001
Model 3[d]	1 [Reference]	0.94 (0.81-1.09)	0.93 (0.80-1.08)	0.76 (0.64-0.90)	.003	0.93 (0.89-0.97)	<.001

Abbreviations: HR, hazard ratio; mPNNS-GS, Programme National Nutrition Santé Guideline Score without the physical activity component; NA, not applicable; Q, quartile.

[a] *P* value for linear trend obtained from the quartile classification by modeling organic food score quartiles as an ordinal variable.

[b] Model 1 is adjusted for age (time scale) and sex.

[c] Model 2 is adjusted for age (time scale) and sex, month of inclusion, occupational status, educational level, marital status, monthly income per household unit, physical activity, smoking status, alcohol intake, family history of cancer, body mass index, height, energy intake, mPNNS-GS, fiber intake, processed meat intake and red meat intake, and (for women) parity, postmenopausal status, use of hormonal treatment for menopause, and use of oral contraception.

[d] Model 3 is model 2 plus further adjustments for ultraprocessed food consumption, fruit and vegetable consumption, and dietary patterns extracted by principal component analysis.

Table 3. Multivariable Associations Between the Organic Food Score (Modeled as Quartiles) and Cancer Risk by Site, NutriNet-Santé Cohort, France, 2009 to 2016[a]

Variable	Q1	Q2	Q3	Q4	*P* Value[b]
Breast cancer, No.	106	115	130	108	NA
HR (95% CI)	1 [Reference]	0.89 (0.68-1.16)	0.93 (0.71-1.20)	0.77 (0.58-1.01)	.10
Premenopausal breast cancer, No.	52	59	66	50	NA
HR (95% CI)	1 [Reference]	1.01 (0.69-1.47)	1.13 (0.78-1.64)	0.89 (0.59-1.35)	.76
Postmenopausal breast cancer, No.	69	55	58	50	NA
HR (95% CI)	1 [Reference]	0.76 (0.53-1.08)	0.75 (0.53-1.07)	0.66 (0.45-0.96)	.03
Prostate cancer, No.	60	47	45	28	NA
HR (95% CI)	1 [Reference]	0.96 (0.66-1.41)	1.12 (0.75-1.66)	1.00 (0.63-1.60)	.78
Colorectal cancer, No.	27	21	27	24	NA
HR (95% CI)	1 [Reference]	0.77 (0.43-1.36)	0.98 (0.57-1.69)	0.87 (0.48-1.57)	.84
Skin cancer, No.	37	35	32	31	NA
HR (95% CI)	1 [Reference]	0.80 (0.50-1.27)	0.69 (0.43-1.12)	0.63 (0.38-1.05)	.06
Non-Hodgkin lymphoma, No.	15	14	16	2	NA
HR (95% CI)	1 [Reference]	0.98 (0.47-2.06)	1.19 (0.57-2.48)	0.14 (0.03-0.66)	.049
All lymphomas, No.	23	16	18	5	NA
HR (95% CI)	1 [Reference]	0.72 (0.38-1.38)	0.87 (0.46-1.65)	0.24 (0.09-0.66)	.02

Abbreviations: HR, hazard ratio; mPNNS-GS, Programme National Nutrition Santé Guideline Score without the physical activity component; NA, not applicable; Q, quartile.

[a] Model is adjusted for age (time scale) and sex, month of inclusion, occupational status, educational level, marital status, monthly income per household unit, physical activity, smoking status, alcohol intake, family history of cancer, body mass index, height, energy intake, mPNNS-GS, fiber intake, processed meat intake and red meat intake, and (for women) parity, postmenopausal status, use of hormonal treatment for menopause, and use of oral contraception.

[b] *P* value for linear trend obtained from the quartile classification by modeling organic food score quartiles as an ordinal variable.

In our study, we observed a lower risk of breast cancer among high organic food consumers. This finding may be interpreted in light of a recent review on the link between breast cancer and various chemicals, which concluded that exposure to chemicals (including pesticides) may lead to an increased risk of developing breast cancer.[53] The inverse association found between NHL and organic food consumption in our study appears to be in line (under the pesticide-harm hypothesis) with a meta-analysis[54] reporting that exposure to malathion, terbufos, and diazinon led to a 22% increased risk of NHL.

Possible underlying mechanistic pathways relating pesticide residues and carcinogenicity include structural DNA damage, as well as functional damage through epigenetic mechanisms. Other mechanisms, such as disorders at the mitochondrion or endoplasmic reticulum level or disturbances of factors implied in maintaining cell homeostasis, are also frequently mentioned.[55] Because endocrine-disrupting pesticides mimic estrogen function, such properties may also be involved in breast carcinogenesis.[56]

When considering different subgroups, the results herein were no longer statistically significant in younger adults, men, participants with only a high school diploma and with no family history of cancer, never smokers and current smokers, and participants with a high overall dietary quality, while the strongest association was observed among obese individuals (although the 95% CI was large). The absence of significant results in certain strata may be associated with lim-

Table 4. Multivariable Associations Between a Simplified Organic Food Score (Modeled as a Continuous Variable and as Quartiles) and Overall Cancer Risk and Cancer Risk by Site, Sensitivity Analyses, NutriNet-Santé Cohort, France, 2009 to 2016[a]

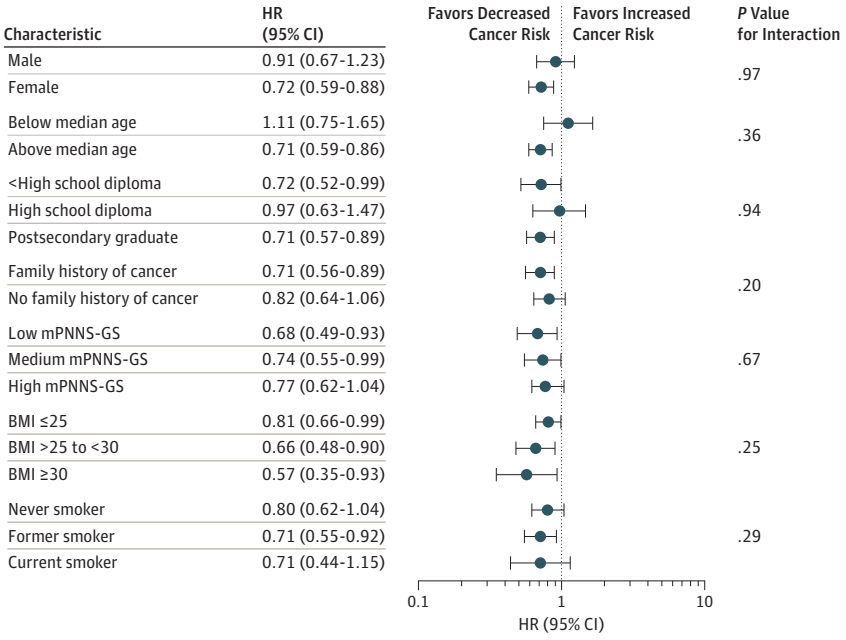
Variable	HR (95% CI)				*P* Value for Trend[b]	HR (95% CI) for a 5-Point Increase	*P* Value
	Q1	Q2	Q3	Q4			
Overall cancer	1 [Reference]	0.94 (0.81-1.09)	0.95 (0.83-1.09)	0.75 (0.63-0.89)	.005	0.92 (0.88-0.96)	<.001
Breast cancer	1 [Reference]	1.06 (0.81-1.39)	1.01 (0.79-1.30)	0.88 (0.66-1.16)	.38	0.95 (0.88-1.01)	.11
Premenopausal breast cancer	1 [Reference]	1.10 (0.75-1.60)	1.14 (0.80-1.61)	1.01 (0.67-1.52)	.85	0.99 (0.99-1.09)	.86
Postmenopausal breast cancer	1 [Reference]	1.03 (0.73-1.45)	0.89 (0.60-1.33)	0.79 (0.53-1.18)	.18	0.91 (0.83-1.01)	.07
Prostate cancer	1 [Reference]	1.14 (0.77-1.68)	1.34 (0.92-1.95)	1.03 (0.61-1.73)	.39	1.02 (0.91-1.15)	.68
Skin cancer	1 [Reference]	0.85 (0.54-1.35)	0.53 (0.33-0.86)	0.79 (0.49-1.28)	.11	0.89 (0.78-1.01)	.06
Non-Hodgkin lymphoma	1 [Reference]	0.80 (0.35-1.81)	1.21 (0.61-2.43)	0.27 (0.07-0.96)	.23	0.75 (0.60-0.93)	.009
All lymphomas	1 [Reference]	0.56 (0.27-1.17)	0.97 (0.54-1.74)	0.23 (0.08-0.69)	.05	0.75 (0.60-0.93)	.03

Abbreviations: HR, hazard ratio; mPNNS-GS, Programme National Nutrition Santé Guideline Score without the physical activity component; Q, quartile.

[a] The simplified organic food score comprises the following plant-derived products that are the main determinants of pesticide exposure: fruits, vegetables, soy-based products, grains and legumes, bread and cereals, and flour. Model (main model) is adjusted for age (time scale) and sex, month of inclusion, occupational status, educational level, marital status, monthly income per household unit, physical activity, smoking status, alcohol intake, family history of cancer, body mass index, height, energy intake, mPNNS-GS, fiber intake, processed meat intake and red meat intake, and (for women) parity, postmenopausal status, use of hormonal treatment for menopause, and use of oral contraception.

[b] *P* value for linear trend obtained from the quartile classification by modeling organic food score quartiles as an ordinal variable.

Figure. Association Between Quartiles of the Organic Food Score (Quartile 4 vs Quartile 1) and Overall Cancer Risk Stratified by Different Factors, NutriNet-Santé Cohort, France, 2009 to 2016



BMI indicates body mass index (calculated as weight in kilograms divided by height in meters squared); HR, hazard ratio; and mPNNS-GS, Programme National Nutrition Santé Guideline Score without the physical activity component.

ited statistical power. Regarding the latter association, previous occupational data have indicated a potential interaction between obesity and pesticide use on cancer risk.[57] It can be hypothesized that obese individuals with metabolic disorders may be more sensitive to potential chemical disruptors, such as pesticides.

Negative associations were observed herein between the risk of cancer and combining both low to medium diet quality and high frequency of organic food consumption. The association between cancer risk and combining both a high-quality diet and high frequency of organic food consumption approached statistical significance. One hypothesis may be that higher intake of pesticide-contaminated products[13] may partly counterbalance the beneficial role of high-quality foods among individuals with a high dietary quality.

While organic food (on confirmation of our findings) may be important to reduce the risk of specific cancers, the high price of such foods remains an important hurdle. Indeed, organic foods remain less affordable than corresponding conventional products, and high prices are a major obstacle for buying organic foods.

Limitations

Some limitations of our study should be noted. First, our analyses were based on volunteers who were likely particularly health-conscious individuals, thus limiting the generalizability of our findings. NutriNet-Santé participants are more often female, are well educated, and exhibit healthier behaviors compared with the French general population.[7,8] These factors may may have led to a lower cancer incidence herein than the national estimates, as well as higher levels of organic food consumption in our sample.

Second, although organic food frequencies in our study were collected using a specific questionnaire providing more precise data than earlier studies, strictly quantitative consumption data were not available. Some misclassification in the organic food score intermediate quartiles herein cannot be excluded.

Third, our follow-up time was short, which may have limited the causal inference, as well as the statistical power for specific sites, such as colorectal cancer. However, more than 1300 cancer cases were registered in this study. The investigation of longer-term effects, while accounting for exposure change, will be insightful as part of the cohort's follow-up. Nevertheless, analyses that were performed by removing cases occurring during the first 2 years of follow-up did not substantially modify our findings.

Fourth, the observed associations may have been influenced by residual confounding. Although we accounted for a wide range of covariates, including major confounders associated with organic food consumption (eg, dietary patterns and other lifestyle factors), residual confounding resulting from unmeasured factors or inaccuracy in the assessment of some covariates cannot be totally excluded. Nutritional covariates, such as the mPNNS-GS or dietary pattern factors, were assessed based on a high level of precision (59 food groups), while the exposure of interest was calculated using a simple scoring method. This may have resulted in a potential bias toward attenuated associations of the exposure of interest. The sensitivity analysis performed applying a simplified, plant-derived organic food score (to account for variations in pesticide exposure across food groups) did not show stringent differences compared with the original organic food score except for breast cancer.

Fifth, we cannot exclude the nondetection of some cancer cases. This is despite the use of a multisource strategy for case ascertainment.

Strengths of our study include its prospective design and the large sample size, allowing us to conduct stratified analyses for different cancer sites. In addition, we used a detailed questionnaire on organic food frequency and clinical validation of cancer cases.

Conclusions

Our results indicate that higher organic food consumption is associated with a reduction in the risk of overall cancer. We observed reduced risks for specific cancer sites (postmenopausal breast cancer, NHL, and all lymphomas) among individuals with a higher frequency of organic food consumption. Further prospective studies using accurate exposure data are necessary to confirm these results and should integrate a large number of individuals. Although our findings need to be confirmed, promoting organic food consumption in the general population could be a promising preventive strategy against cancer.

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Cancer Research

Reduced Levels of IGF-I Mediate Differential Protection of Normal and Cancer Cells in Response to Fasting and Improve Chemotherapeutic Index

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Abstract

Inhibitors of the insulin-like growth factor-I (IGF-I) receptor have been widely studied for their ability to enhance the killing of a variety of malignant cells, but whether IGF-I signaling differentially protects the host and cancer cells against chemotherapy is unknown. Starvation can protect mice, but not cancer cells, against high-dose chemotherapy [differential stress resistance (DSR)]. Here, we offer evidence that IGF-I reduction mediates part of the starvation-dependent DSR. A 72-hour fast in mice reduced circulating IGF-I by 70% and increased the level of the IGF-I inhibitor IGFBP-1 by 11-fold. LID mice, with a 70% to 80% reduction in circulating IGF-I levels, were protected against three of four chemotherapy drugs tested. Restoration of IGF-I was sufficient to reverse the protective effect of fasting. Sixty percent of melanoma-bearing LID mice treated with doxorubicin achieved long-term survival whereas all control mice died of either metastases or chemotherapy toxicity. Reducing IGF-I/IGF-I signaling protected primary glia, but not glioma cells, against cyclophosphamide and protected mouse embryonic fibroblasts against doxorubicin. Further, *S. cerevisiae* lacking homologs of IGF-I signaling proteins were protected against chemotherapy-dependent DNA damage in a manner that could be reversed by expressing a constitutively active form of Ras. We conclude that normal cells and mice can be protected against chemotherapy-dependent damage by reducing circulating IGF-I levels and by a mechanism that involves down-regulation of proto-oncogene signals. *Cancer Res; 70(4); 1564–72. ©2010 AACR.*

Introduction

Most chemotherapy agents cause considerable damage to normal cells, leading to toxicity, which is dose limiting, and causes both short- and long-term side effects in patients. Although drug development has reduced these side effects with a succession of selective antitumor agents, such as antibodies that target specific antigens in cancer or agents with a wider therapeutic index, toxicity continues to limit cancer treatment. Thus, interventions that reduce the undesired toxic side effects could increase the efficacy of many chemotherapy drugs. Chemoprotectants such as amifostine, glutathione, mesna, and dexrazoxane have been investigated and shown to provide drug-dependent protection to specific tissues, but the use of these compounds has not been shown to increase disease-free or overall survival (1). Recently, we reported that short-term starvation (STS) protects normal but not, or much less, malignant cells, leading to improved survival [differential stress resistance (DSR); ref. 2]. Here we present evidence that reduced insulin-like growth factor I (IGF-I) is a major mediator of STS-dependent differential protection.

Biogerontologists have long known that calorie restriction (CR) and/or deficiencies in the pro-growth growth hormone (GH)/IGF-I axis increase stress resistance and life span in various model organisms (3). These beneficial effects can be explained, in part, by the active diversion of energy utilization in starved or IGF-I–deficient organisms. The finite energy source of an organism is finely balanced between growth and maintenance under normal conditions (4). However, under challenging conditions such as starvation, the energy is diverted from growth to maintenance, thereby enhancing protection and survival at the price of growth (5).

During starvation, several changes in the GH/IGF-I axis occur as a result of physiologic adaptation to the new environment. Generally, GH directly regulates the production of IGF-I, which is the major mediator of the growth effects of GH (6). In humans, IGF-I levels decrease dramatically in response to short-term starvation (36–120 hours) despite increased GH secretion (7, 8). Long-lived organisms that are deficient in IGF-I signaling have been shown to be resistant

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to multiple types of stress (9, 10). However, unlike normal cells, cancer cells are self-sufficient in growth signals and insensitive to growth inhibitory signals (11). Self-sufficiency in growth signals is often enabled by gain-of-function mutations in oncogenes [e.g., IGF-I receptor (IGF-1R) or its downstream effectors *Ras, PI3K*, etc.] that result in constitutive activation of proliferation pathways independently or partially independently of external growth factor level. Notably, in normal cells, the RAS/RAF/MAPK and the PTEN/PI3K/AKT pathways can be downregulated by CR or starvation (12), possibly by reducing IGF-I. On the other hand, insensitivity to growth inhibitory signals is due to loss-of-function mutations in tumor suppressor genes (e.g., *Rb, p53, PTEN*, etc.), enabling cancer cells to disregard antiproliferation signals (11, 13). Here we test the hypothesis that the reduction of circulating IGF-I and its signaling mediates the protection of normal cells and mice against chemotherapy toxicity, but not of oncogene-bearing cancer cells, which do not respond to reduced IGF-I.

Materials and Methods

Stress resistance against chemotherapy treatments in LID mice. LID mice, 75 to 100 wk of age, were used to model human cancer treatment. Because liver is the major source of IGF-I production, mice with a conditional hepatic *Igf1* gene knockout have 80% reduced circulating IGF-I levels (14). Because albumin is expressed in the liver after 10 d of birth, resulting in liver *Igf1* gene deletion, LID mice do not experience early death, growth retardation, or developmental defects like the *Igf1* gene knockout ($Igf1^{-/-}$) mice (15). LID and its control mice were given 100 mg/kg etoposide i.v. Cyclophosphamide was given at 500 mg/kg. Cyclophosphamide was dissolved in saline at 40 mg/mL and injected i.p. 5-Fluorouracil (5-FU; Sigma) was injected at 400 mg/kg i.p. Doxorubicin (DXR; Bedford Laboratories) was prepared at 5 mg/mL in saline and injected i.v. first at 20 mg/kg and 22 d later at 28 mg/kg. All drugs have been selected from different categories. All mice were monitored daily for weight loss and signs of pain and stress. Mice determined to be terminally moribund were euthanized by CO_2 narcosis and necropsy was done. Experiments were done in accordance with the Institutional Animal Care and Use Committee (University of Southern California, Los Angeles, CA) and NIH guidelines.

DSR against DXR in LID mice. To study DSR, mice were injected with highly metastatic melanoma cells. LID and its control mice of ages 75 to 100 wk were used. B16Fluc melanoma cells were a generous gift of Dr. Noah Craft at UCLA. B16Fluc cells are derivatives of B16 cells but produce light by stable transfection of the firefly luciferase gene driven by the cytomegalovirus promoter (16). Before injection, cells were washed and resuspended in sterile saline. Each mouse received 2×10^5 cells in 100 μL of saline, followed by another 100 μL of sterile saline to wash off remaining cells in the tails. Three days after tumor inoculation, the first DXR (Bedford Laboratories) injections were given at 16 mg/kg. Two weeks after the initial DXR administration, the second DXR injection was given at 12 mg/kg. Mice were observed daily for signs of stress or pain and body weight was recorded. Mice determined to be terminally moribund were sacrificed by CO_2 narcosis and necropsy was done. The heart was collected for further histologic examination.

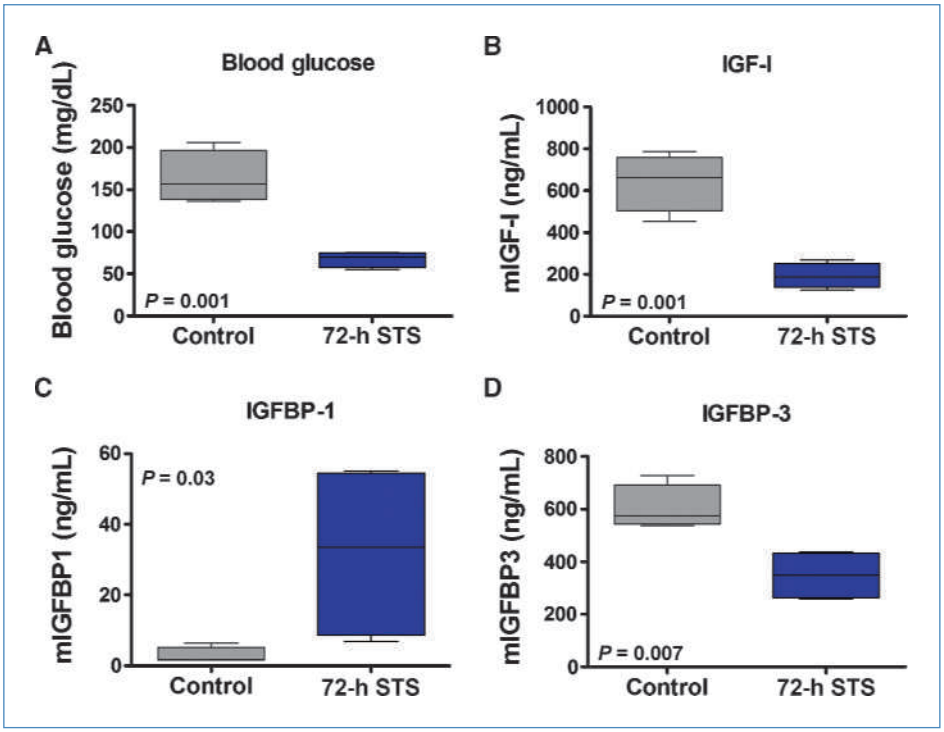

Figure 1. The effect of 72-h fasting on glucose, IGF-I, and IGFBP-1/IGFBP-3 levels. Thirty-week-old CD-1 mice were fasted for 72 h and sacrificed. Blood was collected via cardiac puncture under anesthesia, and blood glucose (A) was measured immediately. Plasma IGF-I (B) and IGFBP-1/IGFBP-3 (C and D) levels were measured by a mouse-specific in-house ELISA. All *P* values were calculated by Student's *t* test except that for IGFBP-1, which was done by the Mann-Whitney *U* test.

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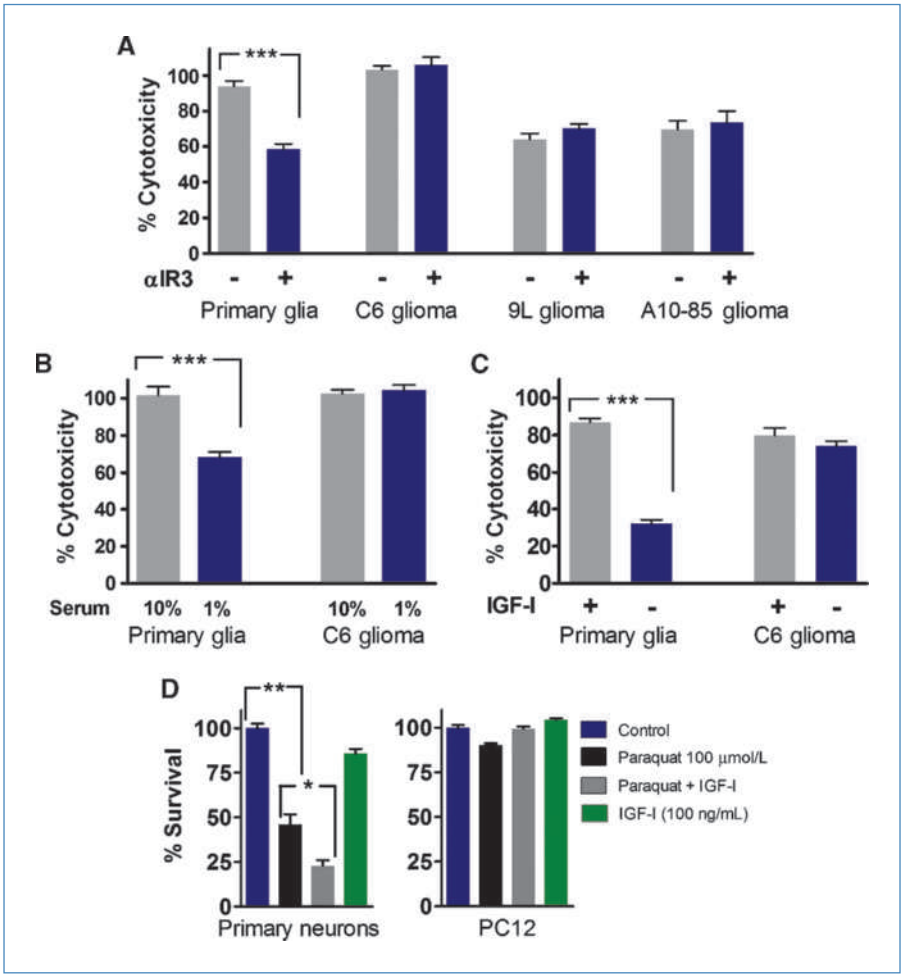

Figure 2. *In vitro* DSR to cyclophosphamide treatments by reducing IGF-I. Primary rat glial cells and rat glioma cell lines (C6, 9L, and A10-85) were tested. A, cells were preincubated in DMEM/F-12 with 1% serum and neutralizing anti–IGF-1R monoclonal antibody α-IR3 (1 μg/mL) for 24 h (15 mg/mL; $n = 12$). B, cells were preincubated in medium with either 1% (STS) or 10% fetal bovine serum for 24 h (15 mg/mL; $n = 12$). C, cells were preincubated in medium with 1% serum with or without rhIGF-I (100 ng/mL) for 48 h (12 mg/mL; $n = 21$). D, effect of IGF-I on DSR against oxidative stress. Chemotherapy drugs such as etoposide, cyclophosphamide, 5-fluorouracil, and DXR have been shown to increase reactive oxygen species and cause oxidative stress. Primary neurons and PC12 pheochromocytoma cells were pretreated with IGF-I (100 ng/mL) for 30 min, followed by paraquat treatments for 24 h. Cytotoxicity (LDH assay) was determined following treatment. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0001$, Student's t test.

Results

Short-term starvation regulates components of the pro-growth GH/IGF-I axis. To investigate the role of the GH/IGF-I axis in the beneficial effects of STS on DSR, we measured the levels of circulating GH and IGF-I and its binding proteins, IGFBP-1 and IGFBP-3, in mice undergoing STS. CD-1 mice were fasted for 72 hours and blood was collected to measure glucose levels and plasma GH, IGF-I, IGFBP-1, and IGFBP-3 levels. After a 72-hour STS, mice had lost approximately 20% of body weight, glucose levels were reduced by 41%, GH levels were slightly increased, and IGF-I levels decreased by 70% (Fig. 1A and B; Supplementary Fig. S1A and B). The bioavailability of IGF-I, which can activate IGF-1Rs, is regulated by IGF binding proteins. In fasted mice, the level of IGFBP-1, which normally reduces IGF-I signaling, increased 11.4-fold (Fig. 1C). These results are in agreement with the reports showing that IGFBP-1 increases in response to fasting in humans and rats (17–19) and also that its overexpression in mice effectively retards growth by sequestering IGF-I (20). Furthermore, the 72-hour fast decreased IGFBP-3 levels by 42% (Fig. 1D), in agreement with reports on short-term fasted humans and rats (19, 21). However, the mechanistic explanation for the decrease in IGFBP-3 is not clear.

To test if restoring the level of IGF-I during STS reverses the protection against chemotherapy toxicity, CD-1 mice underwent a 48-hour STS with IGF-I (200 μg/kg) administration every 12 hours. The level of injected IGF-I was determined from prior serum IGF-I measurements of *ad libitum* fed mice. Following the STS/IGF-I treatments, mice were i.v. injected with 16 mg/kg DXR, a widely used chemotherapy drug acting as an intercalating agent and topoisomerase II inhibitor (22). Indeed, the restoration of IGF-I during STS abolished the protective effect of STS on DXR toxicity, resulting in a 100% versus 38% survival in the STS and STS/IGF-I groups, respectively (Supplementary Fig. S2).

Previously, we showed that primary glia, but not glioma cell lines, preincubated with low glucose (50 mg/dl compared with the normal 100 mg/dl) and low serum (1% fetal bovine serum with the consequent reduction of several growth factors including IGF-I) showed enhanced protection against the alkylating chemotherapy agent cyclophosphamide (2). The glucose levels of fasted mice were reduced to a similar level, along with a dramatic decrease in IGF-I levels (Fig. 1A

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and B). Therefore, the reduction of IGF-I, a potent growth factor, mediates part of the effect of fasting on DSR.

Reduced IGF-I signaling protects primary glia, but not glioma cells, against high-dose cyclophosphamide. IGF-I-like signaling pathways are implicated in regulating life span and stress resistance in organisms ranging from the simple yeast to worms, flies, and mice (3, 23–25). To test the role of IGF-I signaling in DSR against chemotherapeutic drugs *in vitro*, we incubated normal and the equivalent cancer cell lines with an IGF-I receptor (IGF-1R) blocking antibody, low serum concentrations, or excess IGF-I before treatment with cyclophosphamide, a commonly used chemotherapy drug based on its DNA alkylating properties (26). Primary mixed rat glia (astrocytes + 5–10% microglia) and three different rat glioma cell lines (C6, A10-85, and 9L) were tested. All cells were grown to confluence to minimize differences in proliferation rate. Preincubation with an antagonistic IGF-1R antibody (αIR3) protected primary glia, but not the three glioma cell lines, against cyclophosphamide toxicity (Fig. 2A). Reduction of serum level from the standard 10% to 1%, with consequent reduction of growth factors including IGF-I, decreased the toxicity of 15 mg/mL cyclophosphamide to primary glia but not to C6 glioma cells (Fig. 2B). On the other hand, preincubation with 100 ng/mL IGF-I (in the low reference range for adult human serum; ref. 27) caused a 3-fold increase in the toxicity of cyclophosphamide to primary mixed glia but did not increase the toxicity of cyclophosphamide to C6 glioma cells (Fig. 2C). Similar results were obtained with primary neurons and neuron-like pheochromocytoma cells (PC12) treated with a combination of IGF-I and the oxidative stress agent paraquat (Fig. 2D). These results are consistent with our previous studies on fasting and DSR (2) and support the hypothesis that downregulation of IGF-I signaling can protect normal, but not cancer, cells against cytotoxic agents.

Effect of IGF-1R deletion or overexpression on stress resistance in mouse embryonic fibroblast cells. To begin to investigate the mechanism responsible for DSR, we treated mouse embryonic fibroblasts bearing an *Igf1r* deletion (R^- cells) or overexpressing IGF-1R (R^+ cells) with DXR (28). All cells were grown to confluence to minimize the difference in proliferation rate and were treated with DXR for 24 or 48 hours. After a 24-hour DXR treatment, R^- cells showed greater survival compared with R^+ cells. The effect was most pronounced at 25 μmol/L where more than 80% of R^- cells were viable, whereas only 30% of R^+ cells were alive (Fig. 3A; $P < 0.0005$). Similar results were observed when cells were treated for 48 hours, with a 50% versus 12% survival rate for R^- and R^+ cells, respectively, at 25 μmol/L (Fig. 3B; $P < 0.02$).

To further investigate how deficiency in IGF-I signaling protects against chemotoxicity, we measured DNA damage using the comet assay. DXR-induced DNA damage was significantly higher in R^+ cells compared with R^- cells, with a more than 3-fold difference as assessed by the comet assay (Fig. 3C and D; $P < 0.001$). These results support our hypothesis that reduced IGF-I signaling protects normal cells by reducing chemotherapy-dependent DNA damage (29). Notably, R^- cells have been shown to be resistant against transformation by the SV40 large T-antigen, which is remarkable considering that fibroblasts frequently transform in culture spontaneously (30).

The role of yeast homologs of downstream effectors of the IGF-1R in DSR. To investigate the mechanisms by which downregulation of the IGF-1R signaling protects against chemotoxicity and its effect on DNA damage, we turned to the simple model system *S. cerevisiae*. The rationale for using

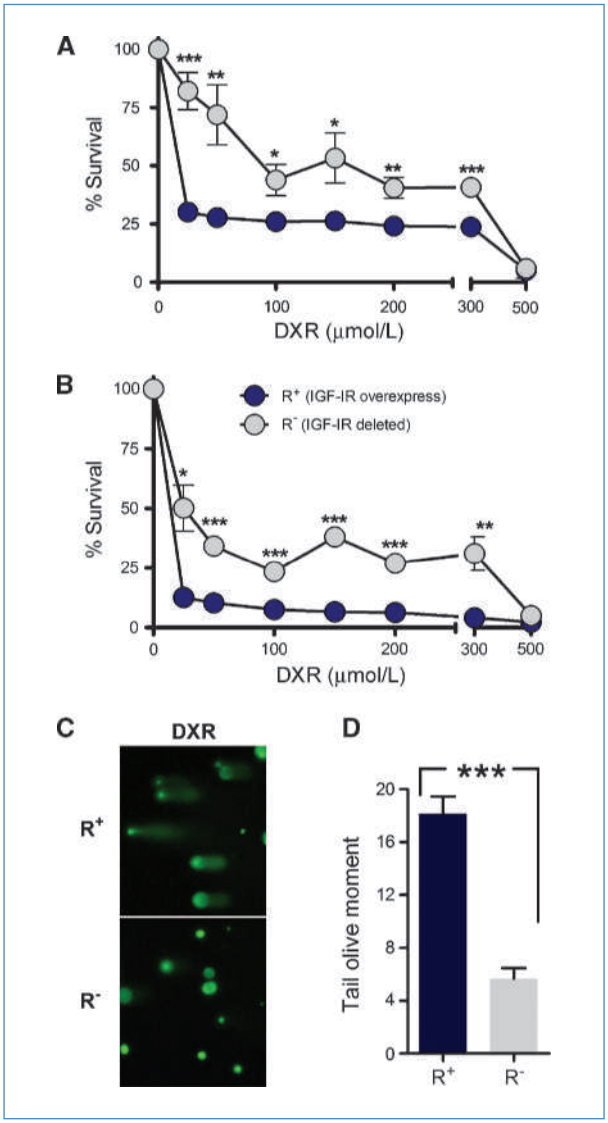

Figure 3. R^+ and R^- cells were grown to confluence and treated with DXR (0–500 μmol/L) in DMEM/F-12 supplemented with 10% fetal bovine serum for 24 h (A) or 48 h (B). Viability was determined by the relative degree of MTT reduction compared with untreated. Points, mean; bars, SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, R^+ versus R^- cells at same DXR concentration (Student's *t* test). C, comet assay. Cells overexpressing IGF-1R or with IGF-1R deficiency (R^+ and R^-) were treated with 50 μmol/L DXR for 1 h. Significant DNA damage was observed in the DXR-treated R^+ cells, whereas R^- cells showed enhanced protection against DXR-induced DNA damage. D, tail olive moment analysis of the comet assay. ***, $P < 0.001$, R^+ DXR versus R^- DXR (Student's *t* test). Similar results were obtained from two independent experiments. A representative experiment is shown.

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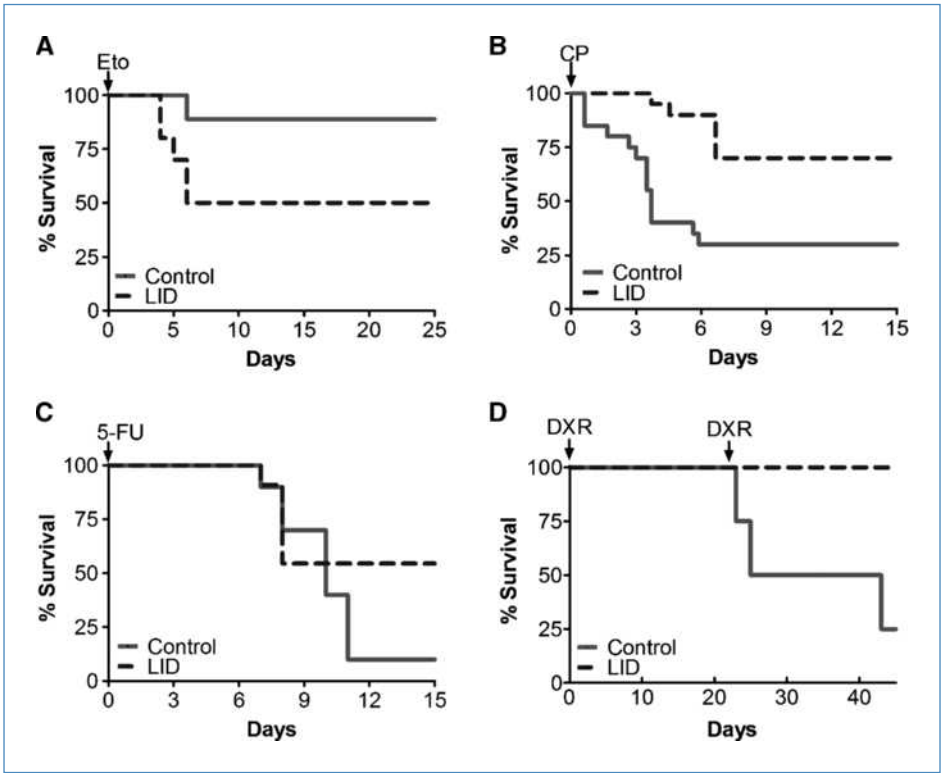

Figure 4. Stress resistance testing in LID mice with various high-dose chemotherapeutic drugs. LID and control mice received (A) a single injection of 100 mg/kg etoposide (n = 10/LID, n = 9/control, P = 0.064), (B) a single injection of 500 mg/kg cyclophosphamide (CP; n = 20/group, P = 0.001), (C) a single injection of 400 mg/kg 5-fluorouracil (n = 11/LID, n = 10/control, P = 0.148), or (D) two injections of DXR. The first injection of 20 mg/kg was given on day 0, and the second injection of 28 mg/kg was given on day 22 (n = 5/LID, n = 4/control, P = 0.022). Toxicity evaluated by percent survival is shown. P values by Peto's log-rank test.

yeast is based on the role of Ras2 and Sch9, homologs of the mammalian Ras and S6K, respectively, in modulating cellular defense against oxidative stress, DNA alkylation, and thermal stress, which was demonstrated in our previous studies (2, 31, 32), and on the central signaling role of homologs of *SCH9* and *RAS2* downstream of IGF-1R (Supplementary Fig. S3A). We tested the effect of the deletion of *RAS2* and *SCH9* on the resistance against DXR. To further investigate DSR, we also studied cells transformed with a gene expressing a constitutively active *RAS2* ($RAS2^{Val19}$) that models human oncogenic Ras mutations. The deletion of *SCH9* (*sch9Δ*) or both *SCH9* and *RAS2* (*sch9Δ ras2Δ*) provided remarkable protection against DXR compared with its wild-type (WT) strains (Supplementary Fig. S3B). However, analogous to what we observed in mammalian cells, the expression of the oncogene-like $RAS2^{Val19}$ reversed the protection provided by *RAS2* and *SCH9* deficiency. After 48 hours of DXR treatment, 50% of WT and $RAS2^{val19}$-expressing cells survived, whereas 70% of *sch9Δ* and more than 90% of *sch9Δ ras2Δ* survived (Supplementary Fig. S3B). The protection was more pronounced after 72 hours of DXR treatment, where *sch9Δ ras2Δ* and *sch9Δ* were highly protected (88% and 70% survival, respectively), but the protection was reversed by the expression of $RAS2^{val19}$ (*sch9Δ* $RAS2^{val19}$; 27% survival). To study the molecular mechanisms of differential resistance to DXR, we monitored DNA mutation frequency, measured as mutations in the *CAN1* gene (Can^r colonies/10^6 cells; ref. 33). DXR treatments increased mutation frequency in all strains. In agreement with the survival analysis, *sch9Δ* and *sch9Δ ras2Δ* exhibited the lowest mutation frequency, whereas $RAS2^{val19}$ expression increased mutation frequency (Supplementary Fig. S3C). The expression of $RAS2^{val19}$ in *sch9Δ* (*sch9Δ* $RAS2^{val19}$) completely reversed the protection provided by the Sch9 deficiency, resulting in a 3-fold increase in mutation frequency (Supplementary Fig. S3B and C). These data suggest that lowered Ras2 and Sch9 signaling has a beneficial effect that could be, at least in part, due to the enhanced protection against DNA damage in the cell, which can be reversed by the expression of oncogenes.

Octreotide sensitizes NXS2 neuroblastoma cells but does not protect mice against high-dose etoposide. Because reduction of IGF-I provided differential chemotherapy protection in mammalian cell culture, we tested if pharmacologic manipulation of the GH/IGF-I axis could induce DSR *in vivo*. The somatostatin analogue octreotide is used in clinics to reduce GH secretion and IGF-I production primarily in acromegaly patients. In addition, octreotide was selected because somatostatin increases in response to fasting (34). Interestingly, octreotide and other somatostatin analogues have been shown to have therapeutic effects in a number of cancers (35) through two distinct effects: direct actions on tumors mediated by somatostatin receptors (36, 37) and indirect effects through inhibition of GH release and the lowering of IGF-I (36–38). In a previous report, we showed that STS provides DSR against high-dose etoposide (2), a widely used chemotherapy drug that inhibits topoisomerase II (39). Here, we tested if inhibiting the GH/IGF-I axis with octreotide could increase the protection against etoposide. We selected a particularly aggressive tumor line (NXS2) that models neuroblastoma (40). I.v. injection

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of NXS2 cells results in a consistent formation of metastasis in multiple organs including the liver, kidneys, adrenal gland, and ovaries (40). A single injection of high-dose etoposide (80 mg/kg) extended the life span of tumor-bearing mice, which otherwise would have succumbed to metastasis within 40 days. In our previous study, STS caused a remarkable reduction in acute chemotoxicity–related deaths, but it also provided partial protection to the cancer cells (2). Our present results indicate that octreotide is not sufficient to protect the animals against chemotherapy, but its combination with STS sensitizes the NXS2 cancer cells to etoposide (Supplementary Fig. S4A and B; Supplementary Fig. S4C, gr. 4 versus gr. 7; $P < 0.01$). However, octreotide had a minimal effect on lowering IGF-I levels in mice (Supplementary Fig. S4D), which could explain its inability to protect the animal. It is possible that homeostatic mechanisms counteract the effect of somatostatin and lead to tachyphylaxis to octreotide treatment, thus failing to reduce IGF-I levels significantly.

To test if octreotide exerted its sensitizing effect on NXS2 cells directly or indirectly, we treated NXS2 cells with octreotide and etoposide *in vitro* (Supplementary Fig. S4E). Octreotide did not alter the toxicity of etoposide to NXS2 cells in cell culture, suggesting that the sensitizing effect of octreotide in mice is indirect. Together, this implies that octreotide alone does not provide starvation-like host protection, but it may reverse the partial protection provided by STS to cancer cells by sensitizing them. Further studies are necessary to investigate the possibility that octreotide may sensitize other tumors against chemotherapy.

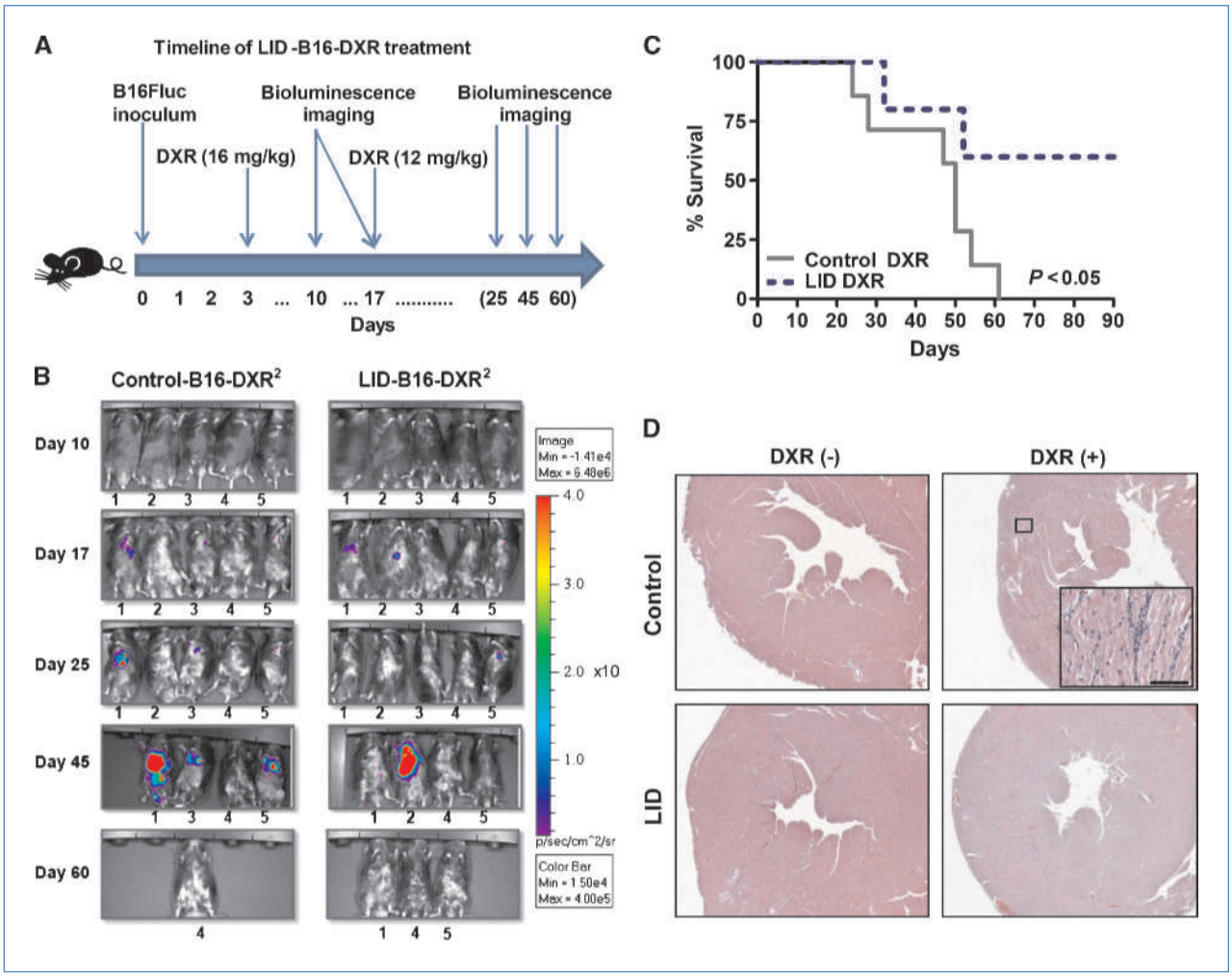

Figure 5. DSR against two cycles of high-dose DXR in melanoma-bearing LID mice. A, timeline of experimental procedures (n = 4/LID-B16, n = 5/LID-B16-DXR, n = 8/control-B16, n = 7/control-B16-DXR). B, bioluminesence imaging of B16Fluc melanoma–bearing LID mice and control mice treated with two cycles of high-dose DXR. Five mice were randomly selected and followed throughout the experiment to monitor tumor progression or regression. C, survival rate comparison between B16Fluc melanoma–bearing LID and control mice treated with two cycles of high-dose DXR ($P < 0.05$). D, DXR-induced cardiomyopathy in control and LID mice. Heart failure is a major outcome of acute DXR toxicity. Histologic slides of the heart from DXR-treated control mice showed loss of myofibrils and infiltration of immune cells, whereas DXR-dependent cardiac myopathy was not observed in LID mice. H&E staining. A representative slide is shown. Bar, 100 μm.

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Enhanced stress resistance in LID mice against high-dose chemotherapy. Mice with genetic disruptions in the IGF-1R or its downstream effectors are more resistant to oxidative stress (9, 41). To determine whether reducing IGF-I signaling protects from chemotoxicity, we tested a transgenic mouse model with a conditional liver *Igf1* gene deletion (LID; ref. 15) resulting in a 70% to 80% postnatal reduction of circulating IGF-I (14), which is similar to that of a 72-hour fasted mouse (Fig. 1B). The LID mice provide a model for investigating the mechanistic relationship between IGF-I and fasting in chemotherapy resistance (42). A single administration of high-dose etoposide led to a 50% versus 88% survival rate, respectively, in the LID and control mice (Fig. 4A; Supplementary Fig. S5A). Based on our *in vitro* results, we tested cyclophosphamide in LID mice. LID mice treated with 500 mg/kg cyclophosphamide showed significantly higher resistance, with a 70% versus 30% survival rate for LID and control mice, respectively (Fig. 4B). Furthermore, whereas LID mice only lost an average of 10% of their weight, control mice lost 20% (Supplementary Fig. S5B). The surviving LID mice also did not show signs of toxicity (Supplementary Fig. S6). To determine the range of protection by reduced IGF-I, we tested two additional drugs, 5-FU and DXR, which represent different classes of chemotherapy drugs. 5-FU is an antimetabolite (43). Survival after a treatment with high-dose 5-FU was improved, with a 55% versus 10% survival rate in LID and controls, respectively, although the difference was not statistically significant (Fig. 4C). Similar but more pronounced effects were observed with DXR. Unlike etoposide and other drugs that can cause irreversible damage to the tail vein of rodents after a single high-dose injection, DXR can be injected for up to two to three cycles (data not shown). To test the effect of multiple cycles of chemotherapy, we challenged LID mice with two cycles of high-dose DXR. The first DXR injection (20 mg/kg) did not result in any toxicity-related deaths, but led to considerable weight loss in all mice (Fig. 4D; Supplementary Fig. S5C). Weight loss was more evident in LID mice during the first 5 days following DXR injection, but unlike the controls that continued to lose weight and showed signs of toxicity, LID mice regained their weight during the following 3 weeks. The second DXR injection (28 mg/kg) caused a considerable amount of DXR-related deaths in the control (25% survival) but not in the LID mice (100% survival; Fig. 4D).

DSR in melanoma-bearing LID mice against high-dose DXR. Next, we tested DSR *in vivo* by monitoring LID mice inoculated with a highly aggressive melanoma cell line (B16Fluc) that metastasizes primarily to the lungs (16) and treating them with DXR. B16Fluc is a luminescent derivative of the B16 mouse melanoma cell line. Therefore, tumor progression and regression can be visualized and quantified *in vivo* using bioluminescence imaging technology (16). LID and its control mice were i.v. injected with B16Fluc (2×10^5 cells per mouse) melanoma cells and treated with two cycles of high-dose DXR (Fig. 5A). Although IGF-I plays a major role in tumor growth and metastasis (44), both LID and its control mice started to succumb to metastasis as early as 25 days following cancer inoculation. The two cycles of high-dose DXR extended survival time by delaying metastasis in all mice (Fig. 5C). Forty-three percent of control mice died with signs of DXR-induced cardiac myopathy, whereas none of the LID mice died from DXR toxicity (Fig. 5D; Supplementary Fig. S7A). In addition, LID mice showed a slight advantage in weight maintenance (Supplementary Fig. S8). Ninety days after cancer inoculation, all control mice that received chemotherapy had died from either cancer metastases or DXR toxicity, but 60% of LID mice that received two cycles of high-dose DXR treatment were cancer-free with no apparent toxic side effects (Fig. 5B and C; Supplementary Fig. S7). All the LID mice deaths were caused by cancer metastases. The progression of cancer between control and LID mice was similar after DXR treatments (Supplementary Fig. S7B), suggesting that the reduction of circulating IGF-I protects the host cells, but not cancer cells, against high-dose chemotherapy.

Discussion

In a previous report, we described a STS-based DSR method to protect the host cells, but not cancer cells, against high-dose chemotherapy. The basis for this seems to be the existence of a nondividing state, which normal cells enter in response to starvation for the purpose of investing the remaining energy resources in cellular protection against various insults. Here, we show that a major reduction in circulating IGF-I can protect the host cells, but not cancer cells, against chemotherapy. Low levels of IGF-I can reduce intracellular mitogenic signaling pathways, including those regulated by Ras and Akt, two of the major pathways downstream of the IGF-1R. We believe that the reduction of mitogenic stimuli may protect normal cells in part by inducing cell cycle arrest (45–47) and in part by shifting the energy toward protection and repair genes such as FOXO, SODs, and DNA repair genes (2, 10, 29, 46, 48). In yeast, we have previously shown that protection can be increased in nondividing cells by up to 1,000-fold, suggesting that a major component of the protective mechanisms is independent of the switch from a dividing to a nondividing state, at least in this simple organism (2). This is also in agreement with the effect of IGF-1R overexpression in sensitizing fibroblasts grown to confluence to DXR (Fig. 3). On the other hand, cancer cells are self-sufficient in growth signals, less or not responsive to physiologic antigrowth signals, and, in many cases, do not undergo cell cycle arrest due to checkpoint dysregulation (11, 29, 47). In fact, it has been shown that pretreatment with nontoxic doses of cell cycle arresting drugs (e.g., DXR) or growth factor inhibitors (inhibitors of MAP/ERK kinase or epidermal growth factor receptor) permits selective killing of checkpoint-deficient cells (49–51).

In support of our hypothesis, our yeast experiments show that deletion of the homologs of *RAS* and/or *SCH9* (AKT/S6K) promotes protection against DXR, but expression of the oncogenic $RAS2^{Val19}$ reverses this cellular and DNA protection independently of cell division. These results raise the possibility that oncogenic mutations that activate pathways such as Ras, AKT, or PKA may reverse the protective effect of reduced IGF-I signaling in malignant cells, thus allowing differential protection of host and various cancers. Notably,

inhibition of the pathway downstream of oncogenic mutations could have either a positive or a negative effect on the protection of cancer cells. Preclinical studies show that IGF-1R targeting strategies can be effective in the treatment of multiple myelomas and prostate, breast, and colon cancers in addition to other cancers (38, 52). The antitumor effect seen with such agents is thought to be dependent on apoptosis resulting from IGF-1R inactivation (52). However, it must be noted that IGF-1R blockade could also trigger apoptosis in normal cells and may not protect against high-dose chemotherapy by interfering with the growth/recovery of certain types of cells (e.g., bone marrow cells). As observed with our LID mice, reduced IGF-I, unlike IGF-1R blockade, does not cause cancer cell death but can selectively enhance the resistance of normal cells against chemotoxicity and may sensitize cancer cells to chemotherapy. This is in agreement with the normal development of prostatic carcinoma in the LID-TRAMP model (14). Based on our results from etoposide-treated LID mice, strategies that reduce circulating IGF-I may also increase the toxicity of certain chemotherapy drugs. Therefore, the compatibility between each drug and IGF-I reduction/blockade therapy should be carefully tested in preclinical studies before being considered as a candidate. Although it seems to be central, IGF-I may represent simply one of a number of growth factors that can activate Ras, Akt, etc., in normal cells and promote cell death in cancer cells and, therefore, only one of the factors that can be downregulated to provide DSR (47).

In summary, our studies in mice indicate that major reduction in circulating IGF-I and in intracellular IGF-I signaling enhances the resistance of the host cells, but not of cancer cells, against chemotherapy, thus providing the foundation for a method to augment cancer treatment without the need to fast. However, the combination of fasting and IGF-I reduction could result in an even more pronounced effect.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Reduced Levels of IGF-I Mediate Differential Protection of Normal and Cancer Cells in Response to Fasting and Improve Chemotherapeutic Index

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