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REVIEW

Role of diet in prostate cancer: the epigenetic link

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Diet is hypothesized to be a critical environmentally related risk factor for prostate cancer (PCa) development, and specific diets and dietary components can also affect PCa progression; however, the mechanisms underlying these associations remain elusive. As for a maturing organism, PCa's epigenome is plastic and evolves from the pre-neoplastic to the metastatic stage. In particular, epigenetic remodeling relies on substrates or cofactors obtained from the diet. Here we review the evidence that bridges dietary modulation to alterations in the prostate epigenome. We propose that such diet-related effects offer a mechanistic link between the impact of different diets and the course of PCa development and progression.

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INTRODUCTION

In the United States, an estimated 233 000 new prostate cancer (PCa) cases will be diagnosed and 29 480 patients will die from PCa in 2014, making this disease the most commonly diagnosed cancer and the second leading cause of cancer-related death in American men.[1] In Europe, PCa is estimated to be the third leading cause of cancer-related death in men for 2014, behind lung and colorectal cancers.[2] There are a few confirmed risk factors for PCa incidence overall, of which age is the most important: PCa is uncommon before 50 years of age and is rarely lethal before 60 years. In fact, 70% of PCa-related deaths occur after age 75.[3] African ancestry and a positive family history are also among the risk factors associated with PCa, and now numerous genetic risk loci have been validated in multiple studies.

The incidence of PCa worldwide can vary by as much as 50-fold between low- and high-risk populations. The large disparity in PCa incidence between the Eastern and the Western hemispheres, a trend observed even before the adoption of prostate-specific antigen testing in developed countries,[4] points to a key role of environmental factors, such as diet, as an etiologic factor in this disease.[5,6] This association is further supported by observations from Japanese immigrants in Los Angeles County, in whom PCa rates are almost quadrupled compared with Japanese living in their homeland and almost match the incidence rate seen in California native residents.[7]

PCa is characterized by complex genomic alterations that are highly heterogeneous and vary greatly from patient to patient, as well as within the same tumor focus. Such disparities can be partly explained by an underlying genomic instability.[8] In addition, PCa has been described as an 'epigenome catastrophe', because various changes in DNA methylation patterns can be detected well before the cancer becomes invasive,[9] suggesting that epigenetic changes are pivotal events in tumor initiation.[10,11] Interestingly, diet can induce various epigenetic modifications that result in global alterations in chromatin packaging; such stable and heritable changes regulate the access of the transcriptional machinery to target genes, and thereby modulate gene expression profiles.[9,12]

Here we introduce some of the evidence that supports the thesis that diet impacts PCa initiation and progression, and examine the hypothesis that these diet-related effects are, in part, mediated by epigenomic alterations.

DIET AND PCa: THE EPIDEMIOLOGICAL EVIDENCE

The impact of diet on cancer growth was first described in landmark studies at the beginning of the 20th century by researchers such as Peyton Rous, who reported that some tumors have a delayed growth and retarded development when transplanted to previously underfed hosts, whereas other tumors are unaffected by the host's diet.[13] We now know that not all cancer types are equally sensitive to dietary modulation,[14] a phenotype that may be attributed in part to defined genetic alterations.[15]

An increasing number of epidemiological and molecular studies point to a link between diet and PCa, particularly for cancers that are more aggressive. Despite this, the role of specific dietary components in PCa development and progression is still unclear. In 2007, the World Cancer Research Fund/American Institute for Cancer Research reported that a diet rich in foods containing lycopene/cooked tomatoes or selenium (nota bene, selenium content in food is mirrored by the soil's selenium abundance) has a protective effect against PCa, whereas diets high in calcium have been associated with increased risk for PCa.[16]

Following this line of reasoning, the role of lycopene and tomato products in PCa prevention has been extensively studied and, although evidence is mixed, available data suggest an inverse association between increased consumption and PCa.[17] In the prospective Health Professionals Follow-up Study, consumption of tomato products was shown to be inversely associated with the incidence of total PCa as well as of advanced stage disease.[18] Also of interest, low levels of selenium have been associated with increased risk of PCa, particularly in relation to advanced or

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aggressive disease.[19] However, selenium supplementation did not significantly reduce the risk of developing PCa in the SELECT randomized trial, indicating that whether selenium intake is obtained directly from the diet or as supplements may impact differently PCa risk.[20] With limited evidence, other potential protective dietary elements include vitamin E, cruciferous vegetables, soy/isoflavones, polyphenols, fish/marine omega-3, coffee and vitamin D.[21–23] Conversely, a number of epidemiological studies have reported an increased risk of PCa for extreme categories of calcium intake,[24] with stronger associations for the risk of advanced or lethal disease.[18] The effect of folate intake (including folic acid supplementation) on PCa risk is conflicting. Although dietary and total folate intake is not associated with PCa risk, high circulating folate levels are associated with an increased risk of PCa,[25] a risk further heightened in patients of African ancestry.[26] With limited evidence, a high dietary intake of red meat and heterocyclic amines, saturated and monounsaturated fats, as well as the essential alpha-linolenic fatty acid (FA) promotes PCa development.[21,23]

FEEDING PCa

Evidence from preclinical models

The impact of diet on PCa progression has been evaluated in various mouse models (see the excellent review by Irshad and Abate-Shen[27] for a detailed overview of the strengths and limitations of each mouse model). It has been shown that a high-carbohydrate/high-fat diet enhances the growth of human PCa cell xenografts in mice.[28,29] In the Hi-Myc transgenic mouse model of PCa, a low-fat diet delays tumor progression,[30] whereas Hi-Myc mice maintained on a calorie-restricted diet display a reduced incidence of *in situ* adenocarcinoma compared with overweight controls (10% kcal from fat) or with mice on a diet-induced obesity regimen (60% kcal from fat).[31] Importantly, calorie-restricted mice do not develop invasive adenocarcinoma, and the frequency of invasive adenocarcinoma is significantly lower in mice fed a low-fat diet compared with mice on the diet-induced obesity regimen. Increased feeding of mice is correlated with greater activation of growth factor signaling,[31] and the greater frequency of prostate adenocarcinoma occurrence in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model has also been attributed to excessive calorie retention.[32] Moreover, a high-fat diet in LADY (12 T-10) transgenic mice is correlated with increased neuroendocrine differentiation, a marker of aggressive PCa.[33]

Similarly, $PTEN^{PE-/-}$ (PE, prostate epithelium) mice that are fed an omega-3 FA-rich diet display reduced PCa growth, slower histopathological progression and increased survival, whereas mice fed on an omega-6 FA-rich diet exhibit the opposite result. Insertion of an omega-3 desaturase (which converts omega-6 into omega-3 FA) into the $PTEN^{PE-/-}$ background rescues the phenotype of mice that are fed the high omega-6 diet.[34] Along the same lines, Yue *et al.*[35] recently observed that esterified cholesterol specifically accumulates in high-grade PCa and metastases, and that this accumulation results from the hyper-activation of the PI3K/AKT pathway following the loss of *PTEN*. Inhibiting acyl-coenzyme A (CoA):cholesterol acyltransferase (*ACAT-1*) results in a net depletion of stored cholesteryl ester, which impedes cell proliferation, migration and even tumor growth in murine xenograft models. Although the underlying mechanism responsible for this unforeseen phenotype, where cholesteryl ester fuels PCa growth, still remains to be fully defined,[35] these observations are further strengthened by the recent findings that ACAT-1 expression can serve as a prognostic marker that readily distinguishes indolent from aggressive PCa.[36]

The human data

In an elegant *ex vivo* study, Aronson *et al.*[37] randomized men with PCa (but not currently under treatment) to either a low-fat (15% kcal) high-fiber and soy-supplemented diet or a typical high-fat (40% kcal) Western diet for 4 weeks; they found that proliferation of LNCaP cells grown in a medium containing 10% human serum from these patients is significantly inhibited only in the presence of serum from men maintained on a low-fat diet for 4 weeks. Consistent with this, obesity is correlated with a lower risk of early stage PCa, as well as an elevated risk of aggressive PCa.[38] In a meta-analysis, Cao and Ma[6] reported that an elevated body mass index of 5 kg/m^2 is associated with a 20% higher PCa-specific mortality. Obesity dysregulates a number of key hormonal pathways and it has been proposed that lower sex hormone-binding globulin, adiponectin and higher insulin, growth hormone, insulin-like growth factor 1 (IGF-1) may also contribute to the development of high-grade tumors in obese patients. In particular, the growth hormone/IGF-1 pathway, known to have a role in the metabolic syndrome (that is, increased blood pressure, high blood sugar level, abnormal cholesterol levels, excess in waist body fat), is implicated in PCa progression.[39–44] Interestingly, high circulating IGF-1 levels are more strongly associated with low-grade than high-grade PCa. This result may reflect a greater dependency of differentiated neoplastic cell on circulating IGF-1 compared with undifferentiated cells that may be less responsive due to a constitutively active PI3K/AKT pathway.[45] In addition, among men diagnosed with PCa in the Physicians' Health Study, excess body weight and a high plasma concentration of C-peptide (a surrogate for insulin levels) both predispose men to an increased likelihood of dying of the disease, further suggesting a role for insulin in PCa progression in obese men.[46] Finally, men with hypercholesterolemia are also more at risk of developing aggressive PCa, a trend reverted by statins' intake.[47]

Collectively, these results obtained from preclinical models and human data demonstrate that both diet and obesity can alter PCa risk and progression. Obviously, the influence of these factors on PCa development is complex and involves a large number of 'classical' signaling pathways (reviewed by Venkateswaran and Klotz[48]). In this review, we propose that diet also alters the prostate epigenome and affects the course of the disease.

THE ALTERED EPIGENOME OF PCa

Epigenetic marks, including DNA methylation and histone modifications, are critical for maintaining a carefully regulated state for the cell. These marks affect local as well as global chromatin packaging, which in turn dictates the sets of active and inactive genes at any given time. It is now clear that cancer development is at least supported,[49] if not initiated,[11] by alterations of the epigenome, which then leads to transcriptional rewiring. Epigenetic modifications observed in PCa evolve throughout disease progression.

DNA methylation in eukaryotes is defined as methylation of the fifth carbon on cytosine residues in CpG dinucleotides (5-methylcytosine). These covalently added methyl groups project into the major groove of DNA and alter transcription.[50] In PCa, genome-wide DNA methylation of cytosine residues in CpG dinucleotides is greatly impaired as the disease progresses to a metastatic stage and leads to global hypomethylation,[51] which can enable the transcription of normally unexpressed proviral and retrotransposon repeats,[52,53] followed by disruption of nearby genes and a predisposition to genomic instability.[53,54] Specific promoter hypomethylation can also reactivate proto-oncogenes such as the urokinase-type plasminogen activator (*PLAU*),[55,56] the matrix metalloproteinase-2 (*MMP2*)[56] or the heparanase (*HPSE*),[57] known to be implicated in tumor invasion and metastasis. On the other hand, promoter hypermethylation and silencing of specific

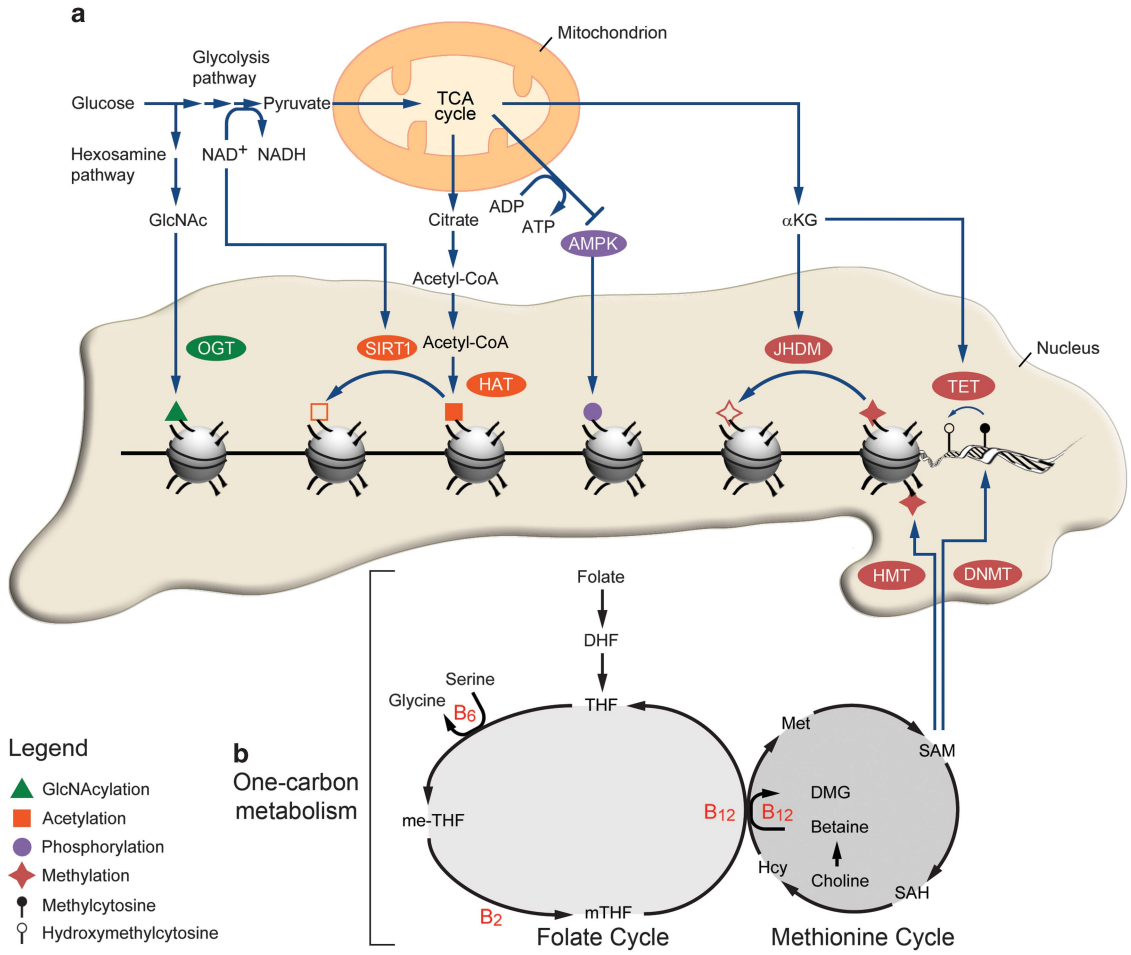

Figure 1. From metabolism to epigenetic remodeling. (**a**) SIRT1 activity depends on the NAD^+/NADH ratio modulated by glycolysis, while *O*-linked *N*-acetylglucosamine transferase uses GlcNAc produced by the hexosamine pathway. Pyruvate entering the tricarboxylic acid (TCA) cycle produces alpha-ketoglutarate, a critical cofactor for Jumonji domain-containing histone demethylase and TET. Acetyl-CoA is converted from the citrate generated by the TCA cycle and used as a donor by histone acetyltransferases. Finally, the increase in ATP/ADP ratio from the TCA cycle also inactivates AMPK. (**b**) SAM acts as a methyl donor for histone methyltransferases and TET and is obtained through the coordinate action of the folate and methionine cycles, termed one-carbon metabolism. αKG: Alpha-ketoglutarate; AMPK: 5′ AMP–activated protein kinase; ADP: adenosine diphosphate; ATP: adenosine triphosphate; B_2: vitamin B_2; B_6: vitamin B_6; B_{12}: vitamin B_{12}; DHF: dihydrofolate; DMG: dimethylglycine; DNMT: DNA methyltransferases; GlcNAc: N-acetylglucosamine; HAT: histone acetyltransferases; Hcy: homocystein; HMT: histone methyltransferases; JHDM: Jumonji domain-containing histone demethylase; OGT: O-linked N-acetylglucosamine transferase; me-THF: 5,10-methylenetetrahydrofolate; Met: methionine; mTHF: 5-methyltetrahydrofolate; NAD+: nicotinamide adenine dinucleotide (oxidized); NADH: nicotinamide adenine dinucleotide (reduced); SAH: S-adenosylhomocysteine; SAM: S-adenosylmethionine; SIRT1: sirtuin histone deacetylase 1; TCA: tricarboxylic acid; TET: ten eleven translocation; THF: tetrahydrofolate.

genes such as that for the detoxification enzyme *GSTP1* is observed in more than 75% of high-grade prostatic intraepithelial neoplasms and in almost all prostate carcinomas (95%),[58] and possibly sensitizes cells to DNA damage. In fact, hypermethylation of the *GSTP1* promoter is a highly specific PCa marker and is rarely detected in benign prostatic hyperplasia[58,59] and normal prostatic tissues.[59,60]

Global patterns of histone acetylation and methylation are also affected throughout PCa progression and can predict the risk of PCa recurrence.[61–63] Bert *et al.*[64] compared the long-range epigenetic remodeling that occurs in different PCa cell lines with that in normal primary cell lines. They used coordinate assessment of histone modifications, DNA methylation profiles and RNA expression; they identified 35 long-range epigenetic activation domains, each about 1 Mb long, and found that a total of 251 genes were activated within these domains—these include oncogenes and genes for microRNAs and PCa biomarkers (for example, *KLK3*, *PCA3*). In particular, alterations of histone marks in PCa cells were characterized either by an enrichment of active histone marks (H3K9ac and H3K4me3) or by the replacement of repressive marks (H3K27me3) by active marks (H3K9ac).[64]

This comprehensive analysis also revealed that, on a genome-wide scale, a subset of long-range epigenetic activation domains were not characterized by promoter hypomethylation, but rather by an extensive DNA hypermethylation in the CpG islands of promoter regions. On the basis of these findings, the authors propose that DNA hypermethylation of promoter regions can prevent the binding of transcriptional repressors, thereby facilitating transcriptional activity.[64] Their findings support a complex interaction between DNA methylation and the histone code in regulating gene transcription.

Together with the report that chromatin modifiers such as *CHD1*, *CHD5* and *HDAC9* are mutated in an important subset of primary PCa,[65] the above results demonstrate that the epigenome undergoes a complex and dynamic remodeling throughout disease progression.

EPIGENETIC MODIFICATIONS AND DIET

A fundamental feature of epigenetic remodeling is its reliance on substrates or cofactors obtained from the diet (Figure 1). When under situations of metabolic stress, the energy-sensing serine-threonine kinase 5′ AMP-activated protein kinase (AMPK) phosphorylates histone H2B at serine 36 and triggers a cell survival program.[66] Histone H2B is also targeted by an *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) residue on serine 112, a glucose-dependent modification that is often located near transcribed genes.[67] The activity of sirtuin histone deacetylase (SIRT) is dictated by the ratio of oxidized and reduced nicotinamide adenine dinucleotide (NAD^+/NADH), which can be modulated by fasting,[68] calorie restriction[69] or dietary supplementation of NAD^+ precursors.[70] Interestingly, in PCa, levels of both NAD^+ and GlcNAc metabolites are altered following seminal vesicle invasion or lymph node metastasis.[71] Alpha-ketoglutarate, an intermediate of the tricarboxylic acid cycle, is also a critical cofactor for histone demethylation by Jumonji domain-containing histone demethylase,[72] as well as for DNA demethylation by ten eleven translocation (Tet) proteins[73] (see the excellent review by Lu and Thompson[74] for details about these metabolite-dependent epigenetic modifications). In addition, the two most well-studied epigenetic processes, namely, methylation and acetylation, are also deeply connected to the diet.

Methylation: an epigenetic modification governed by one-carbon metabolism

DNA and histone methylation by DNA methyltransferases and histone methyltransferases, respectively, requires the transfer of a methyl group (catalyzed by a methyltransferase) from the methyl donor *S*-adenosylmethionine (SAM). Although DNA methylation is usually associated with transcriptional inhibition, the effect of histone methylation depends on the location of the methyl-lysine residue on the histone tail and also on the degree of methylation.[75] SAM is derived from methionine, an essential amino acid that can either be obtained from the diet *per se* or can be generated from homocysteine in a process that utilizes carbon derived from dietary folate, choline or betaine (also a product of choline oxydation) in a vitamin B12-dependent reaction.[76] This cyclic cellular process is termed one-carbon metabolism and is a bicyclic metabolic pathway that refers to the folate and methionine cycles (Figure 1). One-carbon metabolism integrates the donation of carbon units from nutrient inputs into essential cellular processes such as the regulation of redox balance, maintenance of the nucleotide pool, biosynthesis of proteins and the regulation of epigenetic modifications (reviewed by Locasale[77]). Erythrocyte levels of SAM can be altered by dietary intake of fat as well as of calories.[78] Evidence of a link between high serum levels of homocysteine (or deficiency in either folate or vitamin B12) and neural tube defects in the fetus during early stages of pregnancy led to mandatory worldwide folic acid fortification.[79] Finally, because one-carbon metabolism is central to cellular growth and proliferation, folate antagonists—first described in 1948 by Farber and Diamond[80] as a promising treatment for pediatric acute lymphoblastic leukemia—are also used as chemotherapeutic agents.

The yellow agouti (A^{vy}) mouse carries an intracisternal A particle (*IAP*) retrotransposon into the 5′ end of the *agouti* (*A*) gene and is a viable model for determining the impact of diet on epigenetic marks. When unmethylated and active, a cryptic promoter located within the 5′ end of *IAP*'s long terminal repeat hijacks the transcriptional control of the *agouti* gene and leads to ubiquitous expression of the agouti signaling protein; under normal conditions, this protein is restricted to hair cycle-specific patterns.[81] This yields mice that have a yellow coat color and develop multiple health issues such as type II diabetes, obesity and a higher frequency of tumor formation,[82] and serves as a phenotypic readout for a ready assessment of the methylation status of a promoter under different environmental conditions.

A major hallmark of the epigenome is its considerable plasticity during embryogenesis, which enables the differentiation of a single totipotent cell into more than 200 different cell types.[83] Wolff *et al.*[84] published a landmark study in which pregnant non-agouti (*a*/*a*) mothers mated with A^{vy}/*a* males were fed a methyl-supplemented diet (enriched in choline, betaine, folic acid, and vitamin B12), and found that fewer A^{vy}/*a* dams fed *in utero* with the methyl-supplemented diet had a yellow coat color and that this decrease was mirrored by an increased methylation of the A^{vy} proximal long terminal repeat.[85,86] In fact, the darkness of the coat color of the A^{vy}/*a* dams was directly correlated with the degree of methylation of the A^{vy} allele.[87]

In contrast, maternal exposure to bisphenol A (BPA) 2 weeks before mating and throughout gestation and lactation led to an increase in the proportion of A^{vy}/*a* dams that had a yellow coat color and carried a hypomethylated A^{vy} allele. This effect was negated when the BPA diet was supplemented with methyl donors.[88] Alternatively, peri-conceptional feeding of a methyl-deficient diet to female sheep resulted in adult offspring with CpG islands that were hypomethylated or unmethylated relative to animals fed on the control diet. Methyl-deficient diets also led to several health issues, ranging from higher body weight, increased fat, insulin resistance or elevated blood pressure in adult offspring.[89] Similarly, early peri-conceptional exposure to famine during the Dutch Hunger Winter in World War II led to hypomethylation of the imprinted*IGF2* gene in individuals compared with their same-sex siblings, a feature that was maintained for more than 60 years after the event itself.[90] Loss of *IGF2* imprinting is also a feature observed in PCa tissues,[91] as well as in proximal and distal tumor-associated tissues.[92]

Together, these results suggest that dietary modulation of rate-limiting factors of one-carbon metabolism generates long-lasting alterations in the methylation profile, and thus leads to phenotypic changes, in a given organism.

Histone acetylation is a nutrient-sensitive epigenetic mark

Acetylation of lysine residues on histones by histone acetyltransferases neutralizes the basic charge of the lysine, decreases electrostatic affinity between histone proteins and DNA and favors gene transcription via facilitated recruitment of the transcriptional machinery.[93] Lysine acetylation on proteins not only triggers gene transcription, but is also a critical posttranslational modification that regulates the activity of core metabolic enzymes.[94] Analysis of mass spectrometry data reveals that almost every enzyme involved in FA metabolism, glycogen metabolism, glycolysis, gluconeogenesis, the tricarboxylic acid cycle and the urea cycle is acetylated,[95] and functional analysis further documents a complex layer of regulation for protein lysine acetylation of metabolic enzymes. The acetylation status of these metabolic enzymes is responsive to environmental cues—such as the levels of amino acids, FAs or glucose—and modulates the activity and stability of the enzymes.[95]

Fluctuation in protein acetylation in response to dietary factors can be attributed, in part, to the availability of the acetyl group itself, which is obtained from the metabolite acetyl-CoA. Under nutrient-rich conditions, acetyl-CoA is generated by the ATP-citrate lyase (ACL), which catalyzes the conversion of citrate derived from the tricarboxylic acid cycle.[96] Alternatively, acetyl-CoA can be generated through the action of acetyl-CoA synthetases (ACECSs) from the pool of acetate, CoA and ATP. The activity of ACECSs is tightly regulated through reversible acetylation. Under low-nutrient conditions, the NAD^+/NADH ratio increases, activates SIRT1, which in turn de-acetylates and triggers ACECSs activity.[97] Therefore, the pool of acetyl-CoA, which is

governed by nutrient availability, controls the acetylation of metabolic enzymes as well as of histones at any given time.

Along these lines, studies in yeast reveal that levels of acetyl-CoA—which vary depending on the metabolic state—dictate cell growth, in part through the acetylation of histones at growth genes.[98] In yeast, this growth regulation mechanism may be balanced by the competition between histone acetylation and *de novo* FA biosynthesis for the same nucleocytosolic supply of acetyl-CoA, which normally matches growth signals with the required output in macromolecules.[99] In mammalian cells, histone acetylation is similarly dependent on the availability of acetyl-CoA, and inhibiting generation of acetyl-CoA through ACL knockdown thus results in global histone hypoacetylation.[96]

This critical mechanism for regulating cell growth is hijacked by the master transcription factor and proto-oncogene c-Myc, which is implicated in up to 70% of human cancers; Myc overexpression or deregulation results in cancer cells that become addicted to nutrients.[100] Specifically, Myc deregulation leads to the uptake of glucose and glutamine, which are carbon sources used to generate citrate (and consequently acetyl-CoA) through ACL activity.[101] Myc thus increases *de novo* FA biosynthesis and histone acetylation from glucose-derived acetyl groups.[102] Deregulation of cell metabolism by Myc leads to alteration of chromatin structure[103] combined with the generation of the biomass required for supporting uncontrolled cell growth.[104]

PCa: THE IMPACT OF DIET ON THE EPIGENOME

Several studies report a role for dietary components in the remodeling of the cancer epigenome (reviewed by Supic *et al.*[105]). In the context of PCa, the phytoestrogen genistein has the capability to partially demethylate CpG islands in the promoter region of specific genes such as *GSTP1*, leading to increased protein expression.[106] In PCa cell lines, genistein treatment also increases/restores expression of various tumor suppressors including *PTEN*, *p53*, *CYLD, p21WAF1/CIP1* and *p16INK4a*.[107,108] This feature is attributed to the coordinated demethylation and acetylation of H3K9 residues[107] or to increased expression of histone acetyltransferases that result in the enrichment of acetylated histones H3 and H4.[108] Similarly, the flavone apigenin also increases the acetylation of histones H3 and H4 *in vitro* and, when fed orally, significantly impedes PCa tumor growth *in vivo*. In this case, the phenotype is attributed to a marked reduction in histone deacetylase (HDAC) activity as well as in HDAC1 and HDAC3 protein expression.[109] Together, these results suggest that specific dietary molecules can alter PCa progression, in part by remodeling the epigenome. In addition, manipulating the content of dietary methyl donors or dietary fat alters the prostate epigenome and the course of the disease.

Dietary modulation of one-carbon metabolism to influence PCa development

As described above, one-carbon metabolism is central to DNA and histone methylation, as it generates SAM, the ultimate methyl donor. As in earlier studies with use of the A^{vy}/a model,[84] Shabbeer *et al.* used the Hi-Myc mouse model to investigate the impact of excess dietary methyl groups on PCa progression.[110] Overexpression of nuclear Myc protein is frequently detected in prostatic intraepithelial neoplasms, and in a majority of primary carcinomas and metastatic samples,[111] making the Hi-Myc mouse a particularly appropriate mouse model for the study of PCa. Mice were fed a control diet or a 'methyl' diet enriched in choline, betaine, folic acid, vitamin B12 and also in L-methionine and zinc sulfate while *in utero*[112] and during the first month of postnatal life, at which time all mice were fed the control diet. Although given only *in utero* and during early postnatal life, the methyl diet had a long-lasting effect on PCa development. At 5–7 months of age, no invasive adenocarcinoma was detected in prostates from Hi-Myc mice that were fed the methyl diet compared with a high incidence of invasive cancer in the control group. However, this difference in incidence was not observed in younger mice (at 3–5 months of age), suggesting that the methyl diet has an impact on the transition from mPIN to invasive adenocarcinoma, possibly via epigenomic changes.[112] These counterintuitive results indicate that timing might be critical in the context of modulating one-carbon metabolism, and can lead one to hypothesize that the methyl donor diet, if administered during the development of adenocarcinoma, would instead fuel uncontrolled tumor growth by maintaining a hyperactive one-carbon metabolism.

Along the same lines, Bistulfi *et al.* investigated the effects of manipulating dietary folate during disease progression in the TRAMP model, which relies on inactivation of pRb, p53 and PP2A following prostate-specific expression of SV40 large T and small t antigens.[113] TRAMP mice were fed one of three different diets at weaning: a folate-deficient diet, a folate-supplemented diet or a diet containing the recommended amount of folic acid for rodents.[114] Although folate supplementation had little to no effect on tumor growth, folate deficiency clearly improved PCa histopathological parameters compared with the control group, suggesting that folate might be a rate-limiting agent but only when it is under a certain threshold. Depletion of folate from the diet slowed the progression of cancer[114] and the robust arrest of disease progression was attributed by the authors to the secretory function of the prostate, which produces massive amounts of polyamines and exports them into reproductive fluids.[115] Indeed, no reduction in levels of polyamine was found in mice that were fed the folate-deficient diet, although polyamine synthesis draws on pools of SAM through the activity of SAM decarboxylase. This observation suggests that preferential use of SAM for polyamine synthesis under conditions of low folate in the prostate impedes other SAM-related pathways, such as the DNA methylation of CpG islands.[114] Consistent with this, a choline- and methionine-deficient diet led to increased expression of *Igf2* in the prostate of wild-type mice, a result that was mirrored by epigenetic changes at the gene promoter.[116]

In humans, the role of folate in PCa is unclear, although some evidence points to a positive association between high levels of circulating folate and PCa progression.[117] However, before considering the influence on the epigenome of dietary modulation of one-carbon metabolism, it is important to keep in mind that long-term deficiency of dietary methyl donors has important adverse effects. Folate depletion blocks *de novo* biosynthesis of thymidylate, leading to misincorporation of uracil into the DNA and culminating in single-strand DNA breaks[118]—as a consequence, prolonged dietary deficiency of methyl donors in mice leads to the development of intestinal tumors,[119] liver tumors and even to spontaneous mortality.[116] Thus, further experiments aimed at determining the timing, length and extent of a dietary intervention, to effectively impact the course of the disease while keeping side effects to a minimum, are warranted.

The cross talk between lipids and the prostate epigenome

As discussed above, manipulating dietary fat alters the progression of PCa in animal models. In 2010, Llaverias *et al.*[120] showed that increasing both dietary fat and dietary cholesterol significantly accelerates tumor progression in the TRAMP model, but the issue of whether cholesterol *per se* has a role in this aggravated phenotype was left unresolved. Pommier *et al.*[121] attempted to deconvolute these results using a mouse with a double knockout of the genes for the Liver X receptors alpha and beta ($Lxr\alpha\beta^{-/-}$), which encode nuclear receptors central to cholesterol homeostasis. The dorsal prostate lobes of $Lxr\alpha\beta^{-/-}$ mice fed on a standard diet were histologically similar to those of wild-type mice. But when $Lxr\alpha\beta^{-/-}$ mice were fed a high-cholesterol diet,

they accumulated intra-prostatic cholesteryl ester associated with mPIN development; gene expression analysis revealed that two prostatic tumor suppressor genes, *Nkx3.1* and *Msmb*, were downregulated in these mice. This event was attributed to an increase in the H3K27me3 mark at *Nkx3.1* and *Msmb* promoters, possibly a consequence of upregulation of the well-known prostate oncogene histone methyltransferase *Ezh2*.[121,122] Both *LXRβ* downregulation and *EZH2* upregulation have also been reported in human PCa.[123,124] Together with the recent report of abnormal cholesteryl ester accumulation in primary and metastatic human PCa (probably as a consequence PI3K/AKT hyperactivation following *PTEN*-loss),[35] these findings support a role for dietary cholesterol in influencing the prostate epigenome as well as disease progression of PCa.

Aside from dietary cholesterol, *de novo* lipid synthesis may also contribute to the regulation of epigenetic marks, especially histone acetylation. Indeed, *de novo* lipid synthesis is an important hallmark of PCa and correlates with tumor progression and poorer prognosis.[125] Use of an AMPK activator to block *de novo* lipogenesis impedes PCa growth and has been described as a promising treatment avenue, with or without the combined use of AR antagonists.[126] Along these lines, Kee *et al.* demonstrated that overexpression of the enzyme spermidine/spermine N^1-acetyltransferase (SSAT) leads to the diversion of pools of nucleocytosolic acetyl-CoA to polyamine catabolism. In the TRAMP model, overexpression of SSAT leads to a 70% decrease in the availability of acetyl-CoA and resulted in a genitourinary tract that is four times smaller than in control TRAMP mice.[127] It is thus tempting to speculate that *de novo* lipid synthesis observed in PCa also supports cell growth, in part, through global acetylation reprograming.[128]

CONCLUSIONS AND FUTURE DIRECTIONS

Mounting evidence implicates specific diets and dietary components in affecting the course of PCa and the risk of developing the disease. As PCa is considered to be an 'epigenetic catastrophe'[9] and because epigenetic marks rely on substrates or cofactors that are obtained from the diet, we suggest that the impact of diet on PCa development is, at least in part, linked to epigenomic remodeling.

Despite the promising results described here, a number of critical elements remain to be experimentally validated before the causality between diet and the prostate epigenome is established; these include the generation of a comprehensive epigenomic map of both healthy and neoplastic prostatic tissues from different models that are fed on controlled diets, and the metabolomics profile of matching tissues. Such an undertaking would facilitate the determination of the strength of the relationship between diet and the prostate's epigenome. Importantly, results obtained from PCa models should be carefully interpreted relative to their respective oncogenic drivers. Indeed, integrative metabolomic analysis recently revealed that PCa models driven by AKT1 are associated with the accumulation of aerobic glycolysis metabolites, while on the other hand MYC-driven PCa models are associated with dysregulated lipid metabolism.[129] Also, with the emergence of epigenetic-based PCa biomarkers (reviewed by Valdés-Mora and Clark[130]), the identification of common dietary- and cancer-dependent epigenetic alterations could be useful for patient risk stratification as well as for the development of specific dietary guidelines for defined patients.

Recently, epigenetic inhibitors that target DNA methyltransferases (azacitidine, decitabine) or HDAC (vorinostat, romidepsin) have been tested in clinical trials and approved by the US Food and Drug Administration for use in treating defined cancers.[131] Thus, deconvoluting the specific role of diet in rewiring the prostate's transcriptional network may yield critical information and may uncover dietary-related epigenetic pathways that can be therapeutically targeted to prevent or treat PCa.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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