High-fat diet fuels prostate cancer progression by rewiring the metabolome and amplifying the MYC program.

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High-fat diet fuels prostate cancer progression by rewiring the metabolome and amplifying the MYC program

David P. Labbé et al.

Systemic metabolic alterations associated with increased consumption of saturated fat and obesity are linked with increased risk of prostate cancer progression and mortality, but the molecular underpinnings of this association are poorly understood. Here, we demonstrate in a murine prostate cancer model, that high-fat diet (HFD) enhances the MYC transcriptional program through metabolic alterations that favour histone H4K20 hypomethylation at the promoter regions of MYC regulated genes, leading to increased cellular proliferation and tumour burden. Saturated fat intake (SFI) is also associated with an enhanced MYC transcriptional signature in prostate cancer patients. The SFI-induced MYC signature independently predicts prostate cancer progression and death. Finally, switching from a high-fat to a low-fat diet, attenuates the MYC transcriptional program in mice. Our findings suggest that in primary prostate cancer, dietary SFI contributes to tumour progression by mimicking MYC over expression, setting the stage for therapeutic approaches involving changes to the diet.
Prostate cancer is a leading cause of cancer-related lethality. Systemic metabolic alterations can severely affect the course of the disease. Indeed, epidemiological studies have reported that saturated fat intake and obesity are associated with increased prostate cancer progression and mortality. Considering the pandemic of obesity and diet-associated metabolic diseases combined with the high frequency of newly diagnosed prostate cancers in developed countries, a better understanding of the mechanistic underpinnings of this link is of significant importance.

Preclinical and clinical studies have shown that systemic metabolic alterations associated with fat-enriched diets and obesity cooperate with tumour initiating genetic alterations to foster disease progression. Modulation of insulin/insulin-like growth factor 1 levels, phosphatidylinositol-3-kinase/mammalian target of rapamycin complex 1 pathway activation and pro-inflammatory stimuli have been implicated. However, it is now clear that metabolic rewiring is tightly connected to changes at the epigenetic level as metabolites act as substrates or cofactors for epigenetic remodelling.

In prostate cancer, the landscape of epigenetic alterations varies greatly as the disease progresses from a confined tumour to the incurable castration-resistant metastatic stage. However, the influence of metabolic alterations triggered by increased fat intake and/or obesity on prostate cancer epigenome rewiring and disease progression is still unexplored.

The oncogene c-MYC (MYC) is a key driver of human prostate cancer tumorigenesis and progression. MYC protein is overexpressed at early stages of the disease, whereas chromosome 8q gain, or focal amplification of 8q24.21, are associated with amplification of the MYC oncogene in primary prostate cancer, a feature exacerbated in metastatic disease and associated with poor disease-specific survival. In the murine prostate, MYC overexpression faithfully recapitulates the primary human disease.

A hallmark of MYC overexpression in tumours is the induction of a global metabolic reprogramming to support cancer cell survival and growth. Previous studies have shown that increased dietary fat intake significantly alters the biological behaviours of prostate cancers driven by MYC suggesting this preclinical model as ideal to investigate the interplay between HFD, oncogene-driven metabolic vulnerabilities, and epigenetic alterations in prostate cancer progression.

Here, we integrate metabolome, epigenome and transcriptome profiling to identify HFD-driven alterations that foster prostate cancer progression in vivo. We demonstrate that increased fat intake amplifies MYC hallmarks and further enhances MYC’s transcriptional program. Importantly, we identified a fat-induced MYC signature with clinical utility in identifying patients at higher risk of a more aggressive, lethal disease. Altogether, our findings suggest that a substantial subset of prostate cancer patients, including some without MYC amplification, may benefit from epigenetic therapies targeting MYC transcriptional activity or from dietary interventions targeting the metabolic dependencies regulated by MYC.

**Results**

**HFD reprograms cancer metabolome and accelerates progression.** To examine the potential role of high-fat diet (HFD) in promoting metabolic rewiring of prostatic tissues, we compared mice that overexpress a human c-MYC transgene (MYC) in the prostate epithelium to wild-type littermates (WT) that were fed either a HFD (60% kcal from fat; lard—rich in saturated fat) or a control diet (CTD; 10% kcal from fat; Supplementary Table 1). Irrespective of their genotype, mice that were fed with HFD developed the hallmarks of a diet-induced obesity phenotype, including increased body weight, liver steatosis, hyperinsulinemia, hyperglycaemia and a decrease in circulating 1,5-anhydroglucitol (a marker of short-term hyperglycaemia) (Fig. 1a and Supplementary Fig. 1a–e). At 12 weeks of age, MYC overexpression, irrespectively of HFD, resulted in extensive cellular epithelium transformation to prostatic intraepithelial neoplasia (PIN) in the dorsolateral (DLP) and ventral (VP) prostate lobes, the latter with almost complete penetrance. Conversely, the anterior prostate (AP) remained mostly unaffected (Fig. 1b and Supplementary Fig. 1f). No presence of PIN was detected in the prostate lobes of WT animals fed a HFD (Supplementary Data 1). Increased tumour weight (Fig. 1c) and cell proliferation (Ki-67; Fig. 1d) were evident by 36 weeks of age in the HFD-fed mice compared to the CTD group, confirming previous reports that HFD significantly enhances the progression of MYC-driven prostate cancer.

The lack of a HFD-dependent phenotype at 12 weeks of age, combined with the robust and uniform transition to PIN triggered by MYC overexpression observed in the VP (Fig. 1b, c and Supplementary Data 1), enabled us to investigate metabolic alterations driven by HFD before the appearance of a more aggressive, HFD-dependent phenotype. Untargeted metabolomics identified 414 metabolites in the prostate. As previously described, we confirmed that MYC induces a profound metabolic reprogramming in the VP affecting more than half of the metabolites detected, including metabolites related to glutamine, glucose, lipid, nucleotide metabolism and protein synthesis (Fig. 1e–g and Supplementary Data 2). Importantly, we found that these MYC-driven metabolic vulnerabilities were enhanced by HFD. Indeed, HFD resulted in increased levels of metabolites from glycolysis (i.e. lactate), glutaminolysis (i.e. glutamate), glutamine-metabolism related pathways including substrates, intermediates and final products of the citric acid cycle, nucleotide synthesis, amino acid metabolism (e.g. arginine, proline, aspartate and histidine), urea cycle, lipid metabolism and hexosamine biosynthesis (Fig. 1g and Supplementary Data 3); those features were also supported by Metabolite Set Enrichment Analysis (MSEA; Fig. 1h and Supplementary Data 4). Conversely, HFD had little impact on the WT prostatic metabolome, affecting only a total of 12 metabolites, nine of which were glycerophospholipids, and lowering 1,5-anhydroglucitol levels, in line with HFD-driven increase in circulating glucose and reduction of serum 1,5-anhydroglucitol (Fig. 1g, Supplementary Fig. 1d, e and Supplementary Data 5).

Notably, MYC overexpression led to a significant decrease in s-adenosylmethionine (SAM), a member of the methionine cycle and the ultimate methyl donor required for methylation reactions (Fig. 1i). The donation of a methyl group by SAM results in its conversion to s-adenosyldimethylcysteine (SAH), which if accumulated, is a potent inhibitor of methyltransferases. MYC also enhanced the levels of alpha-ketoglutarate (AKG), a critical cofactor for histone demethylation mediated by Jumonji Domain-containing Histone Demethylases (JHDH). Thus, these results suggest that histone methylation processes may be severely hindered during MYC-driven prostate cancer progression. Again, this feature was further exacerbated by diet since increased SAH levels (higher SAH/SAM ratio) were observed in the VP of HFD-fed mice (Fig. 1i and Supplementary Fig. 1h–i). Altogether, our data support the notion that HFD amplifies MYC-driven metabolic reprogramming.

**HFD enhances transcriptional changes at H4K20me1 dynamic genes.** To validate whether MYC/HFD affects histone methylation, we characterised 69 distinct combinations of histone modifications that span H2, H3, and H4 from all four genotype/diet
combinations in all murine prostatic lobes (DLP, VP, AP; Supplementary Data 6) by using a targeted mass spectrometry approach. Unsupervised clustering of the different combinations of histone modifications revealed a strong MYC-driven signature in both DLP and VP (Fig. 2a). This was absent in the AP lobes; unpaired t-test, median, whiskers ± min/max; ****P < 0.0001). b-d HFD does not alter the penetrance of prostatic intraepithelial neoplasia (PIN) at 12 weeks of age (b, n = biologically independent lobes; unpaired t-test, mean ± s.d.; CTD: control diet; AP: anterior prostate; DLP: dorsolateral prostate; VP: ventral prostate; ns: non significant), but does lead to a greater tumour burden (c, n = biologically independent lobes; Welch’s t-test, mean ± s.d.; ***P < 0.0001) and to cell proliferation, as assessed by Ki-67 (d, n = biologically independent lobes; unpaired t-test, median, whiskers ± min/max) in the VP, at 36 weeks of age. e Principal component analysis identifies a distinct metabolic profile in the VP that is triggered by HFD, in a MYC context (n = 6 biologically independent VP/condition, 414 metabolites detected). f, g Representation of all metabolites significantly altered by HFD in a WT (n = 12) or a MYC (n = 89) context, or by MYC overexpression irrespective of the diet (n = 214) (f, unsupervised hierarchical clustering, P < 0.05 and FDR < 0.15); the breakdown of metabolite classes is shown g. h Metabolite Set Enrichment Analysis (MSEA) revealed metabolic pathways significantly enriched by HFD in MYC-transformed VP (P < 0.05 and FDR < 0.15). i Metabolic rewiring triggered by MYC and by HFD in a MYC context suggests dampened histone methylation. Hcy: homocysteine (undetected); Met: methionine; TCA: tricarboxylic acid cycle; Gln: glutamine; Glu: glutamate. Source data are provided as a Source Data file from H4K20me1, a mark that is associated with transcriptional elongation, by the JHDM enzyme PHF8. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) of H4K20me1 revealed highly dynamic levels of this mark along each gene body upon MYC over expression with respect to the corresponding CTD_WT reference (Fig. 2d). Interestingly, modulation of the H4K20me1 mark at the gene body dictates levels of gene expression: thus, loss of H4K20me1 is associated with a decrease, while gain of H4K20me1 is associated with an increase in gene expression (Supplementary Fig. 2c). When comparing the gene expression levels for shared H4K20me1 dynamic gene body-associated regions between CTD_MYC and HFD_MYC conditions (Supplementary Fig. 2d), we found that the MYC-effect was systematically enhanced by HFD (Fig. 2e). These results suggest that HFD further enhances MYC-driven H4K20 hypomethylation leading to transcriptional changes.

High-fat diet enhances MYC transcriptional activity. To determine the cellular program specifically enhanced by HFD
within a MYC context, we performed Gene Sets Enrichment Analyses (GSEA) using the Hallmark gene sets (Supplementary Data 7)\(^3\). As expected, MYC overexpression led to the enrichment of gene sets related to cell proliferation (E2F_targets, related to MYC transcriptional activity (V1/V2), but only in MYC-transformed prostates (Fig. 3a, right). This feature was not linked to an increased expression of the MYC transgene (Supplementary Fig. 3a). Because the MYC transcriptional program is highly context-specific\(^3\), we generated a murine prostatic MYC signature by including the leading edge genes \((n = 610)\) of MYC-related gene sets that were significantly enriched by MYC and/or HFD feeding (Supplementary Fig. 3b and Supplementary Data 8–9). As expected, the expression levels of MYC signature genes were elevated following MYC overexpression, and further increased by HFD (Fig. 3b). ChIP-seq of PHF8, the JHDM that mediates H4K20me1 demethylation\(^3\) and a known MYC transcriptional coactivator and regulator of proliferation\(^3\), revealed that MYC over expression increases the recruitment of PHF8 to the promoter regions of MYC signature genes. Again, we observed that this effect was enhanced by HFD (Fig. 3c).
However, only when MYC over expression was observed in HFD, was a significant decrease in H4K20me1 observed at PHF8 recruitment sites (Fig. 3d, e). Taken together, these results suggest that the observed HFD-induced enhancement of MYC transcriptional program is at least in part, mediated via an increased recruitment and activity of PHF8 toward the H4K20me1 mark at MYC signature genes. This program then culminates in augmented cell proliferation and tumour burden (Fig. 1c, d).

A SFI MYC signature is associated with lethal disease. Since our results in the preclinical model represent the combined effects of both increased dietary animal fat intake (AFI) and the diet-induced obesity phenotype, we next investigated whether dietary AFI, independently of obesity, could recapitulate the HFD-driven phenotype in humans. We used data on AFI, as documented in the Health Professionals Follow-up Study (HPFS) and Physicians’ Health Study (PHS) cohorts, to stratify the 319 prostate cancer patients for whom tumour (genetic background uncharacterised) and adjacent-normal gene expression profiles were available (Table 1). Using GSEA analysis, we identified the MYC_targets_V1 as the three gene sets that were significantly enriched by high AFI, while no gene set was enriched in the adjacent-normal prostatic tissues (Fig. 4a and Supplementary Data 10). When the leading edge genes within the AFI-induced MYC_targets_V1 signature (Fig. 4b, Table 1 and Supplementary Data 11) were used to create a metagene score, we found that prostate cancer patients with greater AFI-dependent MYC transcriptional activation in their tumour tissues were more likely to die of their disease ($n = 31$, Odds Ratio (OR) = 3.44, 95% CI = 1.69–7.38). This holds true after adjusting for gleason grade and body mass index (BMI) (Table 2). We next investigated which type of fatty acid contributes to the enrichment of the MYC transcriptional program. We identified the MYC_targets_V1 as the top gene set that was enriched by high saturated fat intake (SFI; Fig. 4a, and Supplementary Data 12), while neither monounsaturated nor polyunsaturated fat intake was associated with a positive enrichment of any given gene set (Fig. 4a and Supplementary Data 13–14). Importantly, the SFI-induced MYC_targets_V1 signature was more robustly associated with prostate cancer lethality ($n = 34$, Odds Ratio (OR) = 4.02, 95% CI = 1.98–8.63; Tables 1 and 2), a feature that was not recapitulated when using a randomly picked MYC_targets_V1 signature (Supplementary Table 2). Furthermore, the metagene score was more strongly related to lethal disease in men with a high SFI than in men with a low SFI ($P = 0.03$; Fig. 4c). These results indicate that the MYC-transcriptional program specifically induced by SFI drives prostate cancer lethality.

We confirmed the prognostic value of the SFI-induced MYC signature in four independent clinical cohorts by analysing gene expression in the tumours from 631 prostate cancer patients. Strikingly, even in these cohorts lacking patient dietary information, the high expression of the SFI-induced MYC signature identified patients that were more likely to progress to a metastatic disease in the Thomas Jefferson University (TJU), Johns Hopkins Medical Institutions-I (JHMI-I), Mayo Clinic and Cedar-Sinai cohorts ($P = 1.33e-04$), a feature that was much less pronounced when using the non-SFI-associated MYC signature ($P = 1.26e-02$; Fig. 4d). Importantly, in patients from the TJU/JHMI-I/II cohorts, the SFI-induced MYC signature was not associated with BMI (Supplementary Fig. 4). Additional univariate and multivariate analyses confirmed the prognostic power of the SFI-induced MYC signature in predicting prostate cancer progression to a metastatic disease, even after adjusting for gleason grade or the Cell Cycle Progression score consisting of 31 cell cycle genes (Supplementary Tables 3–5)\textsuperscript{37}. Altogether, these
results demonstrate that high SFI, independent of obesity or features of it, fosters a MYC-driven cellular program, promoting the progression to a metastatic and lethal disease.

Finally, we investigated whether a dietary intervention could reverse the HFD-induced MYC transcriptional program. While the HFD robustly enhanced the MYC transcriptional program induced by MYC over expression in the murine prostate, switching to a CTD at 10 weeks of age was sufficient to dampen the MYC transcriptional program. While the progression to a metastatic and lethal disease.

Discussion

In this study, we report the effect of HFD-mediated systemic alterations on prostate cancer progression. Our data demonstrate that HFD synergises with oncogenic transformation of the prostate to promote a MYC-driven program and disease progression. In the normal prostate, HFD impacts metabolites that are primarily restricted to membrane lipid remodelling, has little influence on histone modifications, and results in a distinct transcriptional program compared to that induced by HFD in the transformed prostate. Conversely, HFD profoundly alters an early stage of MYC-induced prostate transformation characterised by PIN, resulting in the enhancement of MYC-driven metabolic, epigenetic, and transcriptional programs (Fig. 4f). These data suggest that a premalignant condition such as PIN, which often precedes the onset of invasive adenocarcinoma in humans,

are primarily restricted to membrane lipid remodelling, has little influence on histone modifications, and results in a distinct transcriptional program compared to that induced by HFD in the transformed prostate. Conversely, HFD profoundly alters an early stage of MYC-induced prostate transformation characterised by PIN, resulting in the enhancement of MYC-driven metabolic, epigenetic, and transcriptional programs (Fig. 4f). These data suggest that a premalignant condition such as PIN, which often precedes the onset of invasive adenocarcinoma in humans, is required for HFD to exert its MYC-amplifying effects in the prostate.

A substantial body of literature supports the notion that cellular metabolism has a profound influence on epigenetic modifications, which rely on metabolites as substrates or cofactors.

Table 1 Characteristics of 319 men diagnosed with prostate cancer from 1982 to 2005 in the Health Professionals Follow-up Study and the Physicians’ Health Study according to fat intake MYC metagene scores

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All men (n = 319)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis, years, mean (SD)</td>
<td>65.0 (6.3)</td>
</tr>
<tr>
<td>Year of diagnosis, n (%)</td>
<td>65.6 (6.2)</td>
</tr>
<tr>
<td>Before 1990 (pre-PSA era)</td>
<td>64.9 (6.6)</td>
</tr>
<tr>
<td>1990-1993 (peri-PSA era)</td>
<td>64.5 (6.1)</td>
</tr>
<tr>
<td>After 1993 (PSA era)</td>
<td>65.6 (6.2)</td>
</tr>
<tr>
<td>BMI at diagnosis, kg/m², mean (SD)</td>
<td>25.2 (2.9)</td>
</tr>
<tr>
<td>PSA at diagnosis, ng/ml, median</td>
<td>7.4</td>
</tr>
<tr>
<td>[25th – 75th percentile]</td>
<td></td>
</tr>
<tr>
<td>T2 NO M0</td>
<td>192 (61.9)</td>
</tr>
<tr>
<td>T3 NO M0</td>
<td>107 (34.5)</td>
</tr>
<tr>
<td>T4/N1/M1</td>
<td>11 (3.5)</td>
</tr>
<tr>
<td>Gleason grade, n (%)</td>
<td>103 (96.3)</td>
</tr>
<tr>
<td>&lt;7</td>
<td>100 (96.3)</td>
</tr>
<tr>
<td>3+4</td>
<td>98 (92.5)</td>
</tr>
<tr>
<td>4+3</td>
<td>96 (91.4)</td>
</tr>
<tr>
<td>&gt;7</td>
<td>5 (4.7)</td>
</tr>
<tr>
<td>Tissue type, n (%)</td>
<td>5 (4.7)</td>
</tr>
<tr>
<td>RP</td>
<td>102 (95.3)</td>
</tr>
<tr>
<td>TURP</td>
<td>99 (93.4)</td>
</tr>
<tr>
<td>Cohort, n (%)</td>
<td>96 (91.4)</td>
</tr>
<tr>
<td>HPFS</td>
<td>5 (4.7)</td>
</tr>
<tr>
<td>PHS</td>
<td>7 (6.6)</td>
</tr>
<tr>
<td>SFS</td>
<td>9 (8.6)</td>
</tr>
<tr>
<td>SD standard deviation, PSA prostate-specific antigen, BMI body mass index, TNM tumour, lymph node, metastasis, RP radical prostatectomy, TURP transurethral resection of the prostate, HPFS Health Professionals Follow-up Study, PHS Physicians’ Health Study.</td>
<td></td>
</tr>
<tr>
<td>*The genes identified in the enrichment analysis of MYC Targets V1 pathway in tumour tissues were used to create a metagene score. A score was computed for each sample by averaging the normalised (mean-centred and variance scaled) expression values of all member genes. The score was divided into tertiles.</td>
<td></td>
</tr>
<tr>
<td>**9 more men missing PSA at diagnosis.</td>
<td></td>
</tr>
<tr>
<td>**Nine men missing pathologic TNM stage.</td>
<td></td>
</tr>
<tr>
<td>**One man missing clinical TNM stage.</td>
<td></td>
</tr>
</tbody>
</table>
A saturated fat-induced MYC signature is associated with lethal prostate cancer. **a**, **b** GSEA analysis (Hallmark) revealed that high animal fat and high saturated fat intake enriches for the MYC_targets_V1 gene set (**a**, \( P < 0.05 \) and FDR < 0.1), as represented by the enrichment plot (**b**) in the HSPH/PHS cohorts. **c** The lethality for every 0.1 unit increase of MYC score was significantly elevated among patients with high saturated fat intake compared with those with low saturated fat intake. **d** High expression of the saturated fat-induced MYC signature is significantly associated with reduced metastasis-free survival (T3) in four independent cohorts (TJU/JHMI-I/Mayo Clinic/Cedar-Sinai cohorts, \( n = 631 \)). **e** Short-term dietary intervention (HFD switch to CTD) dampens the HFD-induced MYC transcriptional activity in MYC-driven murine prostate cancer. **f** Graphical summary.
Table 2 Fat-induced and non-fat-induced MYC signature score in relation to risk of prostate cancer death among men diagnosed with non-metastatic prostate cancer

<table>
<thead>
<tr>
<th>MYC score</th>
<th>n</th>
<th>Leading edge genes (fat-induced)a</th>
<th>OR (95% CI)x</th>
<th>OR (95% CI)y</th>
<th>OR (95% CI)z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 1 (low)</td>
<td>13</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>18</td>
<td>1.58 (0.73, 3.53)</td>
<td>1.30 (0.57, 3.08)</td>
<td>1.27 (0.55, 2.99)</td>
<td>1.00</td>
</tr>
<tr>
<td>Tertile 3 (high)</td>
<td>31</td>
<td>3.46 (1.69, 7.38)</td>
<td>2.50 (1.14, 5.70)</td>
<td>2.37 (1.07, 5.43)</td>
<td>1.00</td>
</tr>
<tr>
<td>P, linear trend</td>
<td></td>
<td>0.001</td>
<td>0.019</td>
<td>0.03</td>
<td>1.00</td>
</tr>
<tr>
<td>Saturated fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 1 (low)</td>
<td>13</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>15</td>
<td>1.23 (0.55, 2.80)</td>
<td>1.07 (0.45, 2.59)</td>
<td>1.05 (0.44, 2.54)</td>
<td>1.00</td>
</tr>
<tr>
<td>Tertile 3 (high)</td>
<td>34</td>
<td>4.02 (1.98, 8.63)</td>
<td>3.21 (1.47, 7.35)</td>
<td>3.04 (1.38, 7.01)</td>
<td>1.00</td>
</tr>
<tr>
<td>P, linear trend</td>
<td></td>
<td>0.0001</td>
<td>0.002</td>
<td>0.004</td>
<td>1.00</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>n</th>
<th>Non-leading edge genes (non-fat-induced)b</th>
<th>OR (95% CI)x</th>
<th>OR (95% CI)y</th>
<th>OR (95% CI)z</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>17</td>
<td>1.00</td>
<td>1.17 (0.57, 2.44)</td>
<td>1.09 (0.49, 2.16)</td>
<td>1.00</td>
</tr>
<tr>
<td>19</td>
<td>1.79 (0.90, 3.64)</td>
<td>1.07 (0.81, 3.70)</td>
<td>1.66 (0.78, 3.61)</td>
<td>1.00</td>
</tr>
<tr>
<td>26</td>
<td>0.09</td>
<td>0.15</td>
<td>0.17</td>
<td>1.00</td>
</tr>
</tbody>
</table>

n = lethal events, OR odds ratio; CI confidence interval
aAnimal fat: n = 122; Saturated fat: n = 113
bAnimal fat: n = 78; Saturated fat: n = 87

Collectively, our results suggest that extrinsic risk factors—such as saturated fat intake—contribute to prostate cancer lethality by enriching for a MYC-transcriptional program; and either synergise with MYC overexpression, which is observed in 37% of metastatic prostate cancers, or phenocopy MYC amplification (Fig. 4f). While neither MYC protein nor MYC mRNA overexpression measured in primary tumours from patients subjected to radical prostatectomy are strong prognostic markers, our findings suggest that a SFI-dependent MYC signature can be used in the clinical setting to identify patients with a worse prognosis. Finally, our study raises the possibility that a nutritional program such as that involving the reduction of animal fat and specifically saturated fat consumption in men with early-stage cancer may dampen the MYC transcriptional program and diminish or delay the risk of disease progression.

Methods

Animal husbandry. FVB-Hi-MYC mice (strain number 01XX8), expressing the human c-MYC transgene in prostatic epithelium, were obtained from the National Cancer Institute Mouse Repository at Frederick National Laboratory for Cancer Research. Upon weaning (3 weeks), male mice heterozygous for the transgene (MYC), together with their wild type littermates (WT), were fed a purified control diet (CTD; Harlan Laboratories, TD. 130838) consisting of 10% fat, or a high-fat diet (HFD; Harlan Laboratories, TD. 06414) consisting of 60% fat (Supplementary Table 1) until 12, 24 or 36 weeks of age; ingredients were adjusted on a kcal basis (Supplementary Table 6). For dietary intervention experiments, mice assigned an HFD were switched to a CTD at 10 weeks of age for the following 2 weeks until the experimental endpoint. Litters were randomly assigned to each diet. Group allocation was performed in a non-blinded fashion. Food was changed on a weekly basis, and mice were weighed every three weeks, starting at weaning. Animals were kept on a 12-h light/12-h dark cycle, and allowed free access to food and water at the Dana-Farber Cancer Institute (DFCI) Animal Resources Facility. The animal protocol was reviewed and approved by the DFCI Institutional Care and Use Committee (IACUC), and in accordance with the Animal Welfare Act. Mice sample size estimate for analyses was based on published literature.

Tissue collection. At defined time points, mice were weighed and euthanized by CO2, followed by cervical dislocation; blood was collected by cardiac puncture, and serum was collected using serum-separating tubes (41.1378.005, Sarstedt), aliquoted, and stored at −80 °C. Urogenital apparatus and liver tissues were fixed in 10% buffered formalin and processed for paraffin embedding. Alternatively, mouse prostate lobes (anterior prostate, AP; dorsolateral prostate, DLP; ventral prostate, VP) were immediately dissected, weighed and flash-frozen in liquid nitrogen. Serum and tissues were consistently collected during the same periods to minimise inter-samples and circadian rhythm variability.

Histopathological and immunohistochemical analyses. Formalin-fixed, paraffin-embedded mouse urogenital apparatus and liver tissues were sectioned (5 μm) and stained with haematoxylin and eosin (H&E). Histopathological slides were analysed by expert murine uropathologist, who were blind to the experimental conditions. Hepatic steatosis was also assessed for liver tissues (M.L.). The presence and extent
of PIN in 12-weeks-old mice (AP, DLP, VP) was estimated for each mouse, by evaluating the percentage of the gland affected for each prostate lobe and reported in Supplementary Table 8. For Ki-67 staining, slides were treated with an antigen retrieval buffer set to 60 °C. They were then loaded into the Bond III staining platform with appropriate labels. Slides were antigen retrieved in Bond Epitope Retrieval 2 for 20 min, and incubated with rabbit monoclonal anti-Ki-67 antibody (#VP-RM04 clone SP6). Vectors Laboratories at dilution 1:250 for 30 min, room temperature. Postantibody washings were performed using Bond Polymer Refine Detection kit. Slides were developed in 3,3’-diaminobenzidine (DAB), dehydrated, and coverslipped. The percentage of Ki-67 positive cells was evaluated by counting the number of cell that expresses nuclear Ki-67 as a function of the total number of cells per high power fields (HPF). Sample size for histological evaluation was estimated based on previous literature data, using the same model. For Ki-67 analysis, we performed sample size calculation using the software G-power version 3.1, extrapolating the effect size (d = 0.87) from the data of Kobayashi et al. in MYC mice fed with HFD. Based on this assumption, we calculated that at least 22 mice/group should be used to detect a significant difference in Ki-67 positivity using a two-sided t-test for change in mean between two independent groups, with an alpha-error of 0.05 and a priori power of 0.8.

**Insulin ELISA.** Serum insulin levels were measured using an insulin-1 ELISA kit from Sigma-Aldrich (#RA0817). Briefly, samples were diluted 1:3 or 1:5 in diluent buffer (including 0.5% BSA) and samples were performed according to the manufacturer’s instructions. Each sample was measured twice (technical duplicate). Outliers (identified using the ROUT method, Q = 0.1%), and samples in which insulin levels were under the detection limit of the assay, were removed from the analysis. Statistical analysis and graphical representation were performed with use of GraphPad Prism version 7.0.

**Metabolic profiling.** For Metabolic profiling of serum and prostatic tissues (VP), we used the platform from Metabolon Inc. (Durham, NC, USA). Mice sample size to ensure adequate power for metabolomics analysis was based on previous literature data using a similar model. Information regarding sample preparation, quality assurance (QA) and control (QC), and metabolite quantification was provided by the company as follows: Sample preparation: Biological samples were stored at –80 °C and then thawed on ice just prior to extraction. Tissue samples were weighed at Metabolon on a 4- position analytical scale (1/10th mg) and then soaked overnight in 80% methanol/ 50% ammonium formate in 50% water, 50% acetonitrile (effective pH 10.60 with NH4OH) (B). The sample injection volume was identical to RP method. The compound mass was then rescaled, to set the median equal to 1. Compounds in which more than 50% of values were missing were not included in the statistical analyses. Scaled data was then rescaled, to set the median equal to 1. Compounds in which more than 50% of values were missing were not included in the statistical analyses. Scaled data were provided in Supplementary Data 2 and 15. Raw and OrigScale data for VP are provided in Supplementary Data 16 and 17. Raw serum data are provided in Supplementary Data 18. These tables include RI, accurate mass values, mean differences in the detected metabolite, and conversion to parts per million (ppm). Metabolomic data were log-transformed (applying the natural logarithm to the data plus one) before data analysis.

*Data analysis: Principal Component Analysis (PCA) using R software was used to visualise the metabolomic data. For PLS-DA, PCA, data were imputed using a k-nearest neighbour (kNN) algorithm (with k = 5); they were then mean-centered and scaled to unit variance. Two-way ANOVA was used to compare the diets (irrespective of genotypes) or genotypes (irrespective of diets) and a t test was used for two groups' comparison (Supplementary Data 2 and Supplementary Data 15). Differences were considered significant if the P value was < 0.05 and to account for multiple testing, a FDR of <0.15. Glucore Omics Explorer (http://www.glucore.com; version 3.1) was used for heatmap representation and unsupervised clustering of metabolites that were significantly altered by HFD in a WT or a MYC context, or by MYC overexpression irrespective of the diet. Metabolites were identified in the 200–800 m/z range (lipids, aminoacids, nucleotides, peptides, carbohydrates, cofactors and vitamins, energy, or xenobiotics), according to Metabolon's classification. Biochemical annotations were assigned by PhD level biochemists at Metabolon, integrating information from literature and public databases (e.g. HMDB). Metabolite Set Enrichment Analysis (MSEA) was performed using a hand-curated metabolite set (Supplementary Table 8). A ThermoFisher Scientific (Waltham, MA) Q-Exactive was the HRAM instrument used. Detailed source and MS settings can be found in Supplementary Table 9 (conditions are also described in supplementary information from Evans et al.33). The scan range was 80–1000 m/z with a scan speed of ~9 scans per second (alternating between MS and MS/MS mode) with a maximum set to 35,000, acquired at 200 m/z. Mass calibration was performed as needed to maintain <5 ppm mass error for all standards monitored.

**Biological sample analysis:** Metabolon has developed a chemometric approach that was used to peak detect and quantification, and is described elsewhere. This in-house peak detection and integration software was used, the data output of which was a list of m/z ratios, retention indices (RI) and area under the curve (AUC) values. User specified criteria for peak detection included thresholds for signal to noise ratio, area and width. Relative standard deviations (RSDs) of peak area were determined for each internal and recovery standard to confirm extraction efficiency, instrument performance, column integrity, chromatography and mass calibration. The biological data sets, including QC samples, were chromatographically aligned based on a retention index that utilised internal standards assigned a fixed RI value. The RI of the experimental peak was determined by assuming a linear fit between known RIs marking whose RIs are set. Peaks were matched against an in-house library of authentic standards and routinely detected unknown compounds specific to the respective method. The library consisted of 3200 endogenous and exogenous metabolites for which super and subpathway designations were provided. Identities were based on retention index values, experimental precursor mass match to the library authentic standard mass (MS/MS match, ~10 ppm, and exact mass match) and MS/MS reverse match scores were based on a comparison of the ions present in the experimental spectrum to the ions present in the library spectrum. A forward score of 100 would mean all the ions present in the experimental spectrum were present in the library at the correct ratios. Any deviations in ion ratios or additional experimental ions observed in the library reduces the forward score, because the forward score is a good indication of the purity of the compound being detected. Co-elution with another molecule with the same mass add ions to the experimental spectrum and reduce the forward score. Similarly, a reverse score of 100 indicated that all ions present in the library were present in the experimental spectrum at the correct ratios and deviations in ion ratios or ions in the library not present in the experimental spectrum reduced the reverse score. Identification was automatically approved if all the above criteria were met and the MS/MS forward and reverse scores were above 80. Compounds which met the above criteria but had low MS/ MS scores, below 35 for both forward and reverse, were automatically rejected. Compounds with intermediate MS/MS forward and reverse scores, 56–79, were marked for manual review. If an MS/MS spectrum was not obtained for a given ion, the identification was based on retention and parent mass alone and marked for analyst reviews. In this case, identification can still be confirmed if it has historical precedent in the specific matrix. Further details can be found in Evans et al.33.

Metabolite quantification and data normalisation: Peaks were quantified using area-under-the-curve. Data was normalised, to correct variations that resulted from differences in the inter-day tuning of the instruments. Essentially, each compound was corrected in run-day blocks, by registering the medians to equal one, and normalising each data point proportionately. Each biological sample was then rescaled, to set the median equal to 1. Compounds in which more than 50% of values were missing were not included in the statistical analyses. Scaled data are provided in Supplementary Data 2 and 15. Raw and OrigScale data for VP are provided in Supplementary Data 16 and 17. Raw serum data are provided in Supplementary Data 18. These tables include RI, accurate mass values, mean differences in the detected metabolite, and conversion to parts per million (ppm). Metabolomic data were log-transformed (applying the natural logarithm to the data plus one) before data analysis.
Global chromatin profiling. The global chromatin profiling assay was performed as described in Creech et al.59, with the following modifications:

Cell lysis. Tissue samples (10–40 mg in mass), were thawed on ice and resuspended in 200 μL ice-cold PBS. Samples were homogenised for about 2 min using a motorised pestle (VWR, 47747–370), and were spun down at 4 °C, at 1500 g for 5 min. Supernatant was removed and 0.5 mL ice-cold nucleic acid buffer was added to the resultant pellet. Nuclei were centrifuged at 4 °C, at 1500 g for 1 min and pellets were washed. The nucleus isolation procedure was repeated twice, removing supernatant each time. Histones were extracted from the remaining pellet, with 400 μL 0.4 N H2SO4 at room temperature for 16 h, while shaking; at this point, histone isolation proceeded using the same protocol as described60. In addition to the flash-frozen tissue, histones were extracted from one 25 million cell pellet of each of Arg-15N2,13C6 SILAC-labeled HeLa, K562, and 293 T (as in Jaffe et al.29), following the protocol described by Creech et al.19.

Histone derivatization. The sample set used SILAC standardisation, with histones extracted from HeLa, K562 and 293T cell lines, as described above. In this workflow, input amount was reduced to 10 μg per sample (3 μg sample and 5 μg SILAC heavy standards), based on the protocol. Samples were adjusted to 100 mM sodium phosphate, pH 8.0, by adding 3 μL 500 mM sodium phosphate, pH 8.0; the total volume of the sample was brought up to 15 μL with HPLC-grade water. Phosphate-buffered samples were reacted with 60 μL of 400 mM NHS propionate in anhydrous methanol at room temperature, with shaking. Three hundred microliters of 0.1% trifluoroacetic acid (TFA) was added, to bring samples to a volumetric concentration of 20% organic solvent. Samples were desalted on a 96-well Oasis HLB 5 mg/cc plate (Waters, 18600309). Activation, equilibration, and wash volumes were 200 μL for each step, and sample elution volume was 100 μL. For the fraction elution, 1 μL trypsin was used in 10 μL of 50 mM ammonium bicarbonate, pH 8.0, while all other conditions were as described59. After digestion and lyophilization, new N-termini were derivatized, by resuspending peptides in 40 μL of 400 mM NHS propionate/anhydrous methanol, and adjusting to 18 mM sodium phosphate, pH 8.0, with 10 μL 100 mM sodium phosphate, pH 8.0. The reaction was quenched by 10 mM 15% hydrogen peroxide for 30 min at room temperature with shaking. Samples were brought up to a total volume of 260 μL with HPLC-grade water, frozen, and lyophilised via vacuum concentrator. Samples were resuspended in 200 μL 0.1% TFA, and desalted on a SepPak C18 96-well μElution plate (Waters, 18602318). All activation and wash volumes were 200 μL. Elution volume was 100 μL. Desalted peptides were lyophilised via vacuum concentrator, and were brought up to a volume of 10 μL with 3% acetonitrile (ACN)/5% formic acid (FA). Samples were further diluted 1:10 with 3% ACN/5% FA, before introducing them into the mass spectrometer.

LC-MS/MS assay parameters: The gradient was modified so that peptides were separated at a flow rate of 200 nL/minute, with a 60 min linear gradient from 97% ACN to 3%. Elution plate (Waters, 18602318). All activation and wash volumes were 200 μL. Elution volume was 100 μL. Desalted peptides were lyophilised via vacuum concentrator, and were brought up to a volume of 10 μL with 3% acetonitrile (ACN)/5% formic acid (FA). Samples were further diluted 1:10 with 3% ACN/5% FA, before introducing them into the mass spectrometer.

Drug-ene. Drug-ene was performed as described in the ChIP-seq Methods section, pooling uniquely indexed RNA-seq libraries from 500 μg of purified total RNA, using TrueSeq Stranded mRNA sample preparation kits (RS-122–2101, Illumina) according to the manufacturer’s protocol; submitted the finished libraries to quality control analyses as described in the ChIP-seq Methods section, pooled uniquely indexed RNA-seq libraries in equimolar ratios, and sequenced to a target depth of 40 M reads on an Illumina NextSeq500 run, with single-end 75 bp reads; Bowtie2 (version 2.2.0) was used to align the ChIP-seq datasets to build version NCBI37/Mm9 of the mouse genome61. Alignment was done with bowtie using default parameters (version 2.2.0). FPKM values were calculated and normalised using the cuffnorm module of cufflinks (version 2.2.0). FPKM values were calculated and normalised using the cuffnorm module of cufflinks (version 2.2.0). Paired t-test was calculated using the t.test function in R (version 3.3.2).

RNA-sequencing. Fresh VP tissues from 12-week-old mice were dissociated to form a single cell suspension. RNA from a similar number of cells was extracted using the miRNeasy Micro Kit (#217084, Qiagen) coupled with on-column DNase treatment (Genta PureLink Micro Kit # KK3485, Kapa Biosystems), according to the manufacturers’ protocols; drug-ene libraries were uniquely indexed in equimolar ratios, and sequenced to a target depth of 40 M reads on an Illumina NextSeq500 run, with single-end 75 bp reads; Fastq files were aligned to the mm9 genome using tophat with default parameters (version 2.0.11). Transcript abundances were calculated using the cuffquant module of Cufflinks (version 2.2.0). FPKM values were calculated and normalised using the cuffnorm module of cufflinks (version 2.2.0).

Murine gene set enrichment analysis and MYC signature. Gene expression values from biological triplicates were input for Gene Set Enrichment Analysis (GSEA)62 using the Hallmark (H, v5.01; Supplementary Data 7) or the Chemical and Genetic Perturbations (C2, z.gnp, v5.1; Supplementary Data 8) Molecular Signature Databases (MSigDB) with 10,000 permutations. The Normalised Enrichment Score (NES)—associated with gene sets that significantly enrich with drug-ene enrichment (P < 0.05 and FDR < 0.1) or the mm9 genome using tophat with default parameters (version 2.0.11). Transcript abundances were calculated using the cuffquant module of Cufflinks (version 2.2.0). FPKM values were calculated and normalised using the cuffnorm module of cufflinks (version 2.2.0). Paired t-test was calculated using the t.test function in R (version 3.3.2).
Protein analysis. Fresh-frozen VP tissues from 12-week-old mice were pulsed (Crysoprep Pulverix, Covaris) and lysed on ice in RIPA buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl. 1 mM EDTA, 1 mM EGTA, 1% NP-40) with the addition of phosphatases and protease inhibitor cocktail tablets (Complete Mini, EDTA-free, Roche). MYC-VP cells were used to map gene names to Affymetrix expression values, and normalised these using the robust multi-array average technical variables and then shifted the residuals to derive the original mean expression values. NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-019-12298-z | www.nature.com/naturecommunications

Statistical analysis: Fat intake after diagnosis was estimated in 4577 men enrolled in the HFPFS and in 926 men from the PHS, all of whom had non-metastatic disease. Predictive utilities were determined by the area under the receiver operating characteristic curve for each cohort, with the highest quintile denoted as the high-fat group and the lower four quintiles grouped as the low-fat group (Supplementary Data 21). The categorised fat intake groups were then integrated with gene expression data in tumour or adjacent normal prostate tissue. Initially, we had 319 tumour tissues from the HFPFS (143 from the HPFS at 100% success rate for the Poly-U probe) and 121 from the HPS for complete fat intake estimation (animal fat: high-fat group n = 65 vs. low-fat group n = 254; saturated fat: high-fat group n = 62 vs. low-fat group n = 257; monounsaturated fat: high-fat group n = 66 vs. low-fat group n = 253; polyunsaturated fat: high-fat group n = 55 vs. low-fat group n = 264) and a total of 157 adjacent normal tissues after merging with fat intake data (animal fat: high-fat group n = 33 vs. low-fat group n = 124; saturated fat: high-fat group n = 29 vs. low-fat group n = 128; monounsaturated fat: high-fat group n = 33 vs. low-fat group n = 124; polyunsaturated fat: high-fat group n = 24 vs. low-fat group n = 133).

Gene set enrichment analysis: Gene expression profiles of tumour and adjacent normal prostate tissues are instinctive for GSEA with use of the Hallmark (H, v4.0) MSigDB with 10,000 phenotype-based permutations, to identify predefined sets of functionally related genes correlated with specific fat intakes (Supplementary Data 10, 12–14). Gene sets with P < 0.05 and FDR < 0.1 were considered for subsequent analyses. Animal fat and saturated fat intake-dependent MYC (n = 139) and JP6 was obtained by combining either the leading or the non-leading edge genes from the MYC_targets_V1 gene set from the H MSigDB in tumour tissues (Supplementary Data 11), to create a metagene score as previously described59. This was computed for each sample by averaging the normalised (mean-centered and variance scaled) expression values of all member genes. An additional signature was derived from 132 from random genes from the MYC_targets_V1 gene set (Supplementary Data 11). Odds ratios and 95% confidence intervals were obtained by logistic regression for the association between the metagene score and lethal prostate cancer. The score was modelled as categorical (tertiles). We tested for linear trend across score categories by modelling the log-transformed continuous variable. All models were adjusted for age and year at diagnosis. We further adjusted for Gleason grade to test whether the score is an independent predictor of lethal prostate cancer and BMI at diagnosis, to differentiate the effect from overweight/obesity. To assess whether the association between the score and lethal prostate cancer was modified by saturated fat intake, we obtained interaction terms including an interaction term (saturated fat intake x MYC score) in the multivariable model using a Wald test. All analyses were conducted using SAS version 9.3 and R version 3.1.0.

Validation cohorts: To investigate the power of SFI-induced and non-SFI-induced MYC signatures to predict metastatic disease, we utilised genome-wide expression profiles of 731 patients with metastatic outcome follow-up from the Decipher Genomic Resource Information Database (GRID; NCT02669269). These patients were pooled from four studies of either case-cohort or cohort design. Patients for these studies came from four institutes: Thomas Jefferson University (n = 199)65, Johns Hopkins Medical Institutions (n = 253; polyunsaturated fat: high-fat group n = 23 vs. low-fat group n = 124; polyunsaturated fat: high-fat group n = 24 vs. low-fat group n = 133).

Data of statistical analyses. All the statistical tests were justified as appropriate. Assumption criteria were met, analysis of variance was performed. When the F ratio (test statistic) was not equal to 1 (i.e., unequal variance was applied). Data are reported including estimation of variation within each group. Two-sided tests were used. Measurements were taken from distinct samples.
The sequencing data reported in this paper (ChIP-seq and RNA-seq) were deposited on MetaCon and are available through the study identifier MTBLS135. Raw, scaled metabolomics data, and statistics were also provided as supplementary tables. The sequencing data reported in this paper (ChIP-seq and RNA-seq) were deposited on MetaCon and are available through the study identifier MTBLS135. Raw, scaled metabolomics data, and statistics were also provided as supplementary tables. The sequencing data reported in this paper (ChIP-seq and RNA-seq) were deposited on MetaCon and are available through the study identifier MTBLS135. Raw, scaled metabolomics data, and statistics were also provided as supplementary tables.

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Author contributions

Additional information
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Competing interests: C.Y.L. receives sponsored research from and consults for Kronos Bio, is a shareholder and inventor of IP licensed to Syros Pharmaceuticals, is a shareholder of Amgen, and is an equity partner of Cambridge Science Corporation. M.A., N.E., M.T., I.L., E.A.G. and E.D. are employees of Decipher Biosciences. E.D.K. is currently employed of Metabolon. M.B. receives sponsored research support from Novartis. M.B. is a consultant to Alera Biotherapeutics and J3 Biomedicine and serves on the SAB of Kronos Bio. The remaining authors declare no competing interests.

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