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

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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 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 over expression, 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), gluconolysis (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 (MSA; Fig. 1h and Supplementary Data 4).

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, g 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-adenosylhomocysteine (SAH), which if accumulated, is a potent inhibitor of methyltransferases. MYC also enhanced the levels of alpha-ketoglutarate (αKG), a critical cofactor for histone demethylation mediated by Jumonji Domain-Containing Histone Demethylases (JHDH)28. 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...
Combination of 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 (Supplementary Fig. 2a) in line with the marginal PIN penetration observed in this lobe (Fig. 1b). Among the histone peptides monitored, H3K27/K36 and H4K20 were significantly affected by MYC overexpression. As previously described, MYC overexpression induced a steep decrease in H3K27me3 (corresponding to the H3K27me3K36meX peptides). In particular, the H3K27me3 mark was hypomethylated in a stepwise process that can be catalysed by multiple JHDM enzymes and culminates with the unmethylated/acetylated H3K27 mark (Supplementary Fig. 2b). A similar pattern was observed for the H4K20 mark, but in this case the effect of MYC was significantly enhanced by HFD, leading to greater levels of the unmethylated mark (Fig. 2b). Importantly, HFD had no effect on the H4K20 mark in the WT tissues (Fig. 2b, c). H4K20me0 can be generated 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 overexpression 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, 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 over expression, 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).

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**Fig. 2** High-fat diet enhances MYC-driven transcriptional changes at H4K20me1 dynamic genes. a Global chromatin profiling identifies distinct chromatin-signature profiles in MYC-overexpressing DLP and VP lobes (histone marks levels relative to the DLP, VP and AP CTD_WT median values; MYC vs. WT comparisons, unpaired \(t\) test; \(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\); Supplementary Data 6). b HFD enhances H4K20 hypomethylation triggered by MYC (MYC vs. WT and HFD_MYC vs. CTD_MYC comparisons, unpaired \(t\) test; \(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\)). c H4K20me0 levels relative to the CTD_WT condition (fold change; unpaired \(t\) test, mean ± SEM; \(P < 0.01\), ***\(P < 0.001\), ****\(P < 0.0001\)). d H4K20me1 dynamic at gene body

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However, only when MYC over expression was assessed with 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 lethal = 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). Conversely, when we used the MYC signature genes not enriched by AFI (non-leading edges genes) to create a similar metagene score, this score was unable to predict lethal disease after adjusting for Gleason grade and 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 lethal = 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 for interaction = 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)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, thereby reducing or delaying the progression to a lethal, metastatic 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, 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. Here, we provide the evidence that HFD acts as a master effector of prostate cancer metabolism, creating an environment that favours histone hypomethylation and results in an enhanced MYC-driven transcriptional program. Notably, we observed a decrease in the H4K20m1 Pr mark at the promoter region of MYC signature genes, a feature that was associated with both an increased recruitment and activity of PHF8, a JHDM that relocates to the promoter to coact on the MYC gene in response to HFD.

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

| Characteristic                  | All men (n = 319) | Animal fat MYC metagene scorea | Saturated fat MYC metagene scorea |
|--------------------------------|------------------|-------------------------------|----------------------------------|
|                                | (n = 107)        | Tertile 1 (low)               | Tertile 2 (n = 106)             | Tertile 3 (high) (n = 106)      |
| Age at diagnosis, years, mean (SD) | 65.0 (6.3) | 65.6 (6.2) | 64.9 (6.6) | 64.5 (6.1) | 65.6 (6.2) | 64.7 (6.3) | 64.7 (6.3) |
| Year of diagnosis, n (%)       | 6                     | 28 (8.8) | 9 (8.4) | 10 (9.4) | 9 (8.5) | 8 (7.4) | 12 (11.3) | 8 (7.5) |
| Before 1990 (pre-PSA era)      | 83 (26.0) | 32 (29.9) | 27 (25.5) | 24 (22.6) | 32 (29.9) | 27 (25.5) | 24 (22.6) |
| After 1993 (PSA era)           | 208 (65.2) | 69 (65.1) | 69 (65.1) | 73 (68.9) | 67 (62.6) | 67 (63.2) | 74 (69.8) |
| BMI at diagnosis, kg/m², mean (SD) | 25.2 (2.9) | 24.7 (2.6) | 25.4 (2.9) | 25.5 (3.1) | 24.7 (2.6) | 25.4 (2.8) | 25.6 (3.2) |
| PSA at diagnosis, ng/ml, median | 7.4        | 7.0       | 7.6       | 8.0       | 6.9       | 7.6       | 7.9       |
| [25th – 75th percentile]b | [5.3-11.6] | [5.0, 11.9] | [5.5, 12.9] | [5.6, 11.1] | [5.0-12.0] | [5.4-13.0] | [5.6-11.1] |
| Pathologic TNM stage, n (%)c  |                       |                       |                       |                       |                       |                       |                       |
| T2 NO M0                      | 192 (61.9) | 73 (69.5) | 60 (58.3) | 59 (57.8) | 72 (68.6) | 60 (58.3) | 60 (58.8) |
| T3 NO M0                      | 107 (34.5) | 29 (27.6) | 40 (38.8) | 38 (37.3) | 30 (28.6) | 40 (38.8) | 37 (36.3) |
| T4/N1/M1                      | 11 (3.5)   | 3 (2.9)   | 3 (2.9)   | 5 (4.9)   | 3 (2.9)   | 3 (2.9)   | 5 (4.9)   |
| Clinical TNM stage, n (%)d    |                       |                       |                       |                       |                       |                       |                       |
| T1/T2 NO M0                   | 297 (93.4) | 103 (96.3) | 98 (92.5) | 96 (91.4) | 102 (95.3) | 99 (93.4) | 96 (91.4) |
| T3 NO M0                      | 21 (6.6)   | 4 (3.7)   | 8 (7.5)   | 9 (8.6)   | 5 (4.7)   | 7 (6.6)   | 9 (8.6)   |
| Gleason grade, n (%)           |                       |                       |                       |                       |                       |                       |                       |
| <7                             | 51 (16.0)   | 24 (22.4) | 16 (15.1) | 11 (10.4) | 22 (20.6) | 17 (16.0) | 12 (11.3) |
| 3–4                           | 124 (38.9)  | 49 (45.8) | 39 (36.8) | 36 (34.0) | 49 (45.8) | 40 (37.7) | 35 (33.0) |
| 4–7                           | 81 (25.4)   | 19 (17.8) | 32 (30.2) | 30 (28.3) | 20 (18.7) | 32 (30.2) | 29 (27.4) |
| Tissue type, n (%)             |                       |                       |                       |                       |                       |                       |                       |
| RP                            | 63 (19.7)   | 15 (14.0) | 19 (17.9) | 29 (27.4) | 16 (15.0) | 17 (16.0) | 30 (28.3) |
| TURP                          | 31 (97.4)   | 105 (98.1) | 103 (97.2) | 103 (97.2) | 105 (98.1) | 103 (97.2) | 103 (97.2) |
| Cohort, n (%)                 |                       |                       |                       |                       |                       |                       |                       |
| HPFS                          | 8 (2.5)     | 2 (1.9)   | 3 (2.8)   | 3 (2.8)   | 2 (1.9)   | 3 (2.8)   | 3 (2.8)   |
| PHS                           | 213 (66.8)  | 61 (57.0) | 73 (68.9) | 79 (74.5) | 65 (60.7) | 68 (64.2) | 80 (75.5) |
|                                | 106 (33.2)  | 46 (43.0) | 33 (31.1) | 27 (25.5) | 42 (39.3) | 38 (35.8) | 26 (24.5) |

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, PHFS Physicians’ Health Study

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

[44x25]the MYC_targets_V1 signature observed in 12-week-old mice

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Fig. 4 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.
genetic heterogeneity of human prostate cancers.\textsuperscript{19,44} Indeed, dietary fat intake does not only amplify the MYC transcriptional program in MYC-driven prostate cancers, but can enrich for it, even in cancers lacking MYC overexpression. This suggests that the enhancement of MYC-driven metabolic and epigenetic reprogramming may be a general mechanism that underlies the influence of dietary fat intake on prostate cancer progression although this hypothesis remains to be tested across prostate cancer molecular subtypes.

Finally, we show that the saturated fat-induced MYC transcriptional signature is not only a tumour biomarker for the patients’ diet, but it is prognostic for progression to metastatic, lethal prostate cancer. Indeed, the SFI-induced MYC signature is able to predict prostate cancer lethality, independently of the degree of tumour differentiation and patient obesity status and the robustness of this finding was validated in four independent cohorts. Importantly, the non-fat-induced MYC signature was unable to predict lethal disease in the HPFS/PHS cohorts and was only marginally significant as prognostic marker in the validation cohorts. This highlights the fact that saturated fat intake not only enriches the expression of MYC-regulated genes but does so especially for the most predictive subset of genes, possibly reflecting the convergence of oncogenic signalling pathways on dysregulated lipid metabolism, a key feature for prostate cancer development and progression to a metastatic disease.\textsuperscript{45}

Several in vitro studies showed that MYC drives the de novo synthesis of fatty acids and increases the expression of the key lipogenic enzymes such as the ATP Citrate Lyase (ACLY), Acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN)\textsuperscript{46–48}. Moreover, previous metabolic profiling performed by our group on MYC engineered prostate cells, in vivo models, and human prostate cancers showed that MYC overexpression specifically enhances lipid metabolism underlying lipid deregulation as key metabolic feature of MYC oncogenic activity in prostate cancer\textsuperscript{26}. Recently HFD has been shown to induce an intra-tumoural lipogenic signature driving metastasis formation in \textit{Pten} deficient mouse model\textsuperscript{49}. We are currently performing lipidomics analysis to investigate the interplay between diet-derived fats and MYC-driven de novo lipogenesis in shaping the tumour lipodome and promoting a more aggressive phenotype.

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 over expression, which is observed in 37% of metastatic prostate cancers\textsuperscript{50}, 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\textsuperscript{51}, 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\textsuperscript{31}. 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 was in accordance with the Animal Welfare Act. Mouse sample size estimate for analyses was based on published literature.

#### Tissue collection

At defined time points, mice were weighed and euthanized by \textit{CO}2 followed by cervical dislocation; blood was collected by cardiac puncture, and serum was collected using serum-separating tubes (Nalgene 411378.005, Sarstedt), aliquoted, and stored at $-80^\circ$C. Urogenital apparatus and liver tissues were fixed in 10% buffered formalin and processed for paraffin embedding. Alternatively, mouse prostate lobes (anterior prostate, AP; dorsosateral prostate, DLP; ventral prostate, VP) were immediately dissected, weighted and flash-frozen in liquid nitrogen. Serum and tissues were consistently collected during the same periods to minimise inter-samples and circadian rhythm variability.

#### Histopathologic 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

### 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

| MYC score | n | Leading edge genes (fat-induced)\textsuperscript{a} | Non-leading edge genes (non-fat-induced)\textsuperscript{b} |
|-----------|----|--------------------------------|---------------------------------|
| Animal fat |     | OR (95% CI)\textsuperscript{c} | OR (95% CI)\textsuperscript{d} |
| Tertile 1 (low) | 13 | 1.00 | 1.00 |
| Tertile 2 | 18 | 1.58 (0.73, 3.53) | 1.27 (0.55, 2.99) |
| Tertile 3 (high) | 31 | 3.46 (1.69, 7.38) | 2.37 (1.07, 5.43) |
| P, linear trend\textsuperscript{d} | 0.001 | 0.019 | 0.03 |
| Saturated fat |     | OR (95% CI)\textsuperscript{c} | OR (95% CI)\textsuperscript{d} |
| Tertile 1 (low) | 13 | 1.00 | 1.00 |
| Tertile 2 | 15 | 1.23 (0.55, 2.80) | 1.05 (0.44, 2.54) |
| Tertile 3 (high) | 34 | 4.02 (1.98, 8.63) | 3.04 (1.38, 7.01) |
| P, linear trend\textsuperscript{d} | 0.0001 | 0.002 | 0.004 |

\textsuperscript{a}Estimated by modeling tertiles of MYC score as continuous variable (tertile 1 = 0, tertile 2 = 1, tertile 3 = 2)
\textsuperscript{b}Logistic regression model adjusted for age and year at diagnosis (continuous)
\textsuperscript{c}Logistic regression model adjusted for age and year at diagnosis (continuous), gleason grade (continuous: <7, 3 ≥7)
\textsuperscript{d}Logistic regression model adjusted for age and year at diagnosis (continuous), gleason grade (continuous: <7, 3 ≥7), and BMI at diagnosis (continuous)
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 1 (CL). For Ki-67 staining, slides were immersed in xylene for 15 min and then in an oven 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 (¾VP-RM04 (clone SP6), Vectors Laboratories) at dilution 1:250 for 30 min, room temperature. Primary antibody was detected using the polymer-based detection kit. Slides were developed in 3,3′-diaminobenzidine (DAB), dehydrated, and coverslipped. The percentage of Ki-67 positive cells was counted by counting the number of cell that expresses nuclear Ki-67 as a function of the total number of cells in high power fields (HPF). Wherever possible, up to 10 high power fields for each VP lobe were counted, averaged, and counts were reported as each sample’s score (F.G. and M. F.). Sample size for histological evaluation was estimated based on previous literature data, using the same model10. 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.13 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 (#AR80187). Briefly, samples were diluted 1:3 or 1:5 in diluent buffer (1:3 dilution) and the assay was 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). Mouse sample size was chosen to ensure adequate power for metabolomics analysis was based on previous literature data, using the same model10. 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.13 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.

Statistical analysis was performed with use of GraphPad Prism version 7.0.

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Global chromatin profiling. The global chromatin profiling assay was performed as described in Creech et al.59, with the following modifications:

**Heatmap generation**

**GENE-E** (http://www.broadinstitute.org/cancer/software/GENE-E) was used for heatmap representation as well as statistical analysis of the data, using the comparative marker selection suite.60 Differences were considered significant if the p-value was <0.05, and FDR was <0.1. Unsupervised clustering of heatmap data was performed using the VennDiagram R package (version 1.6.9).
Protein analysis. Fresh-frozen VP tissue from 12-week-old mice were pulverised (Cryosprep Pulverizer, 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-CP cells (kindly provided by Dr. Charles Sawyer, Memorial Sloan Kettering Cancer Center, New York, NY) 

were rinsed on ice with PBS and lysed as for the mouse prostate tissues. Cell lysates were harvested via Trizol (ThermoFisher) and 1 μg of total RNA was used for cDNA synthesis using the High Capacity cDNA Reverse transcription kit (ThermoFisher). cDNA was hybridised to a GeneChip Human Gene 1.0 ST array (Affymetrix). The array was scanned and expression values were normalised using the robust multi-array average (RMA) method. The data was deposited in the GEO database under accession number GSE21051.

Statistical analysis. Gene 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, and normalised these using the robust multi-array average technical variables and then shifted the residuals to derive the original mean 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, and normalised these using the robust multi-array average technical variables and then shifted the residuals to derive the original mean 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, and normalised these using the robust multi-array average technical variables and then shifted the residuals to derive the original mean expression values, and normalised these using the robust 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Author contributions
D.P.L., G.Z., M.L. and M.B. conceived the study and designed the experiments. Wet lab experiments were performed by D.P.L., G.Z. and A.L.C. and supported by H.E., S.S., L.E., J.D.I., M.L. and M.B. E.D.K. performed MS-based metabolomics analysis. Computational analyses were performed by D.P.L., M.Y., J.M.R., C.Y.L., S.C., M.A. and N.E. and supported by E.M.E., A.G., M.T., I.L., E.D., A.Y.D., P.W.K., I.E.B., L.A.M., J.E.C., M.L. and M.B. Access to validation cohorts data was provided by A.R., E.M.S., R.B.D., R.I.K., S.F.F. and D.E.S. Pathological analyses were performed by F.G., M.F. & M.L. D.P.L., G.Z., M.Y., S.C., A.L.C., M.L. and M.B. interpreted the data and drafted the paper considering inputs from all co-authors.

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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 Aloita Biotherapeutics and H3 Biomedicine and serves on the SAB of Kronos Bio. The remaining authors declare no competing interests.

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