New Prostate Tumour Marker

PRK1 controls Tumour Growth

Albert-Ludwigs-Universität Freiburg

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Technology

Protein-kinase-C-related kinase 1 (PRK1) phosphorylates histone H3 at threonine 11 (H3T11). PRK1 knockdown or inhibition impedes androgen receptor-dependent transcription. Blocking PRK1 function abrogates androgen-induced H3T11 phosphorylation. Importantly, levels of PRK1 and phosphorylated H3T11 correlate with Gleason scores of prostate carcinomas. Finally, inhibition of PRK1 blocks proliferation of androgen receptor-induced tumor cell proliferation, making PRK1 a promising therapeutic target.

Innovation

- better treatment of prostate tumor
- PRK1 and H3T11ph are markers for tumor progression

Application

- Modulation of PRK1 activity is tissues where AR has a pivotal physiological role i.e.:
- Treatment of prostate tumor
- Control of fertility
- Treatment of Alzheimer´s disease
- Treatment of Parkinson´s disease

Market Potential

- Prostate cancer represents the most frequent malignant disease in men worldwide and the second leading cause of death from malignant tumors.

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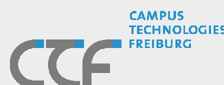

Phosphorylation of histone H3 at threonine 11 establishes a novel chromatin mark for transcriptional regulation

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Abstract

Posttranslational modifications of histones such as methylation, acetylation and phosphorylation regulate chromatin structure and gene expression. Here we show that protein-kinase-C-related kinase 1 (PRK1) phosphorylates histone H3 at threonine 11 (H3T11) upon ligand-dependent recruitment to androgen receptor target genes. PRK1 is pivotal to androgen receptor function because PRK1 knockdown or inhibition impedes androgen receptor-dependent transcription. Blocking PRK1 function abrogates androgen-induced H3T11 phosphorylation and inhibits androgen-induced demethylation of histone H3. Moreover, serine-5-phosphorylated RNA polymerase II is no longer observed at androgen receptor target promoters. Phosphorylation of H3T11 by PRK1 accelerates demethylation by the Jumonji C (JmjC)-domain-containing protein JMJD2C. Thus, phosphorylation of H3T11 establishes a novel chromatin mark for gene activation, identifying PRK1 as a gatekeeper of androgen receptor-dependent transcription. Importantly, levels of PRK1 and phosphorylated H3T11 correlate with Gleason scores of prostate carcinomas. Finally, inhibition of PRK1 blocks proliferation of androgen receptor-induced tumour cell proliferation, making PRK1 a promising therapeutic target.

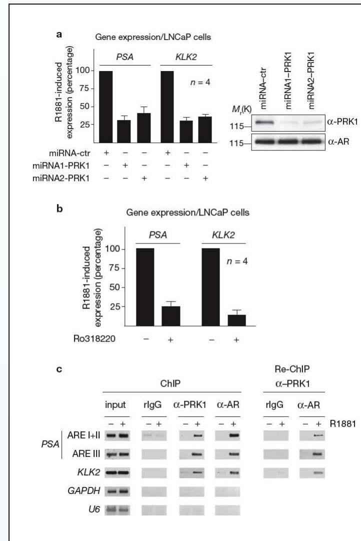

Figure 1 - PRK1 controls androgen receptor (AR)-dependent gene expression and associates with chromatin. LNCaP cells were cultivated in the presence or absence of the androgen receptor agonist R1881. miRNA-mediated PRK1 knockdown (a) or the PRK1 inhibitor Ro318220 (b) reduce expression of the endogenous PSA and KLK2 genes (a, left panel, b). Western blot analysis (a, right panel) verified the specific miRNA-mediated knockdown of PRK1 (bars represent mean + s.d., n = 4). ChIP and Re-ChIP (c) using the indicated antibodies demonstrate androgen dependent association of androgen receptor and PRK1 at promoters of androgen receptor-regulated genes. The precipitated chromatin was amplified by PCR using primers flanking the AREs in the promoter region of the PSA and KLK2 genes, or the promoters of the unrelated GAPDH and U6 genes.

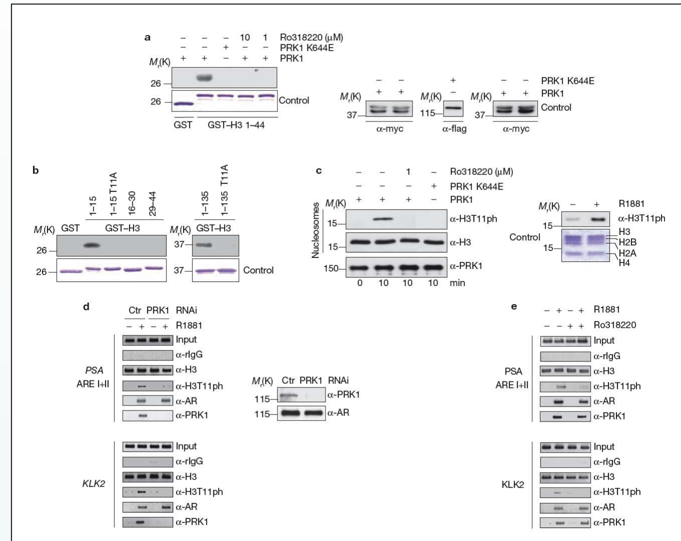

Figure 2 - PRK1 phosphorylates histone H3 at threonine 11 (H3T11). Bacterially expressed GST and GST–H3 (a, b) or nucleosomes from HeLa cells (c, left panel) were incubated for the indicated time with active PRK1 or the kinase dead mutant PRK1 K644E in the presence or absence of the inhibitor Ro318220. Coomassie blue staining (a and b, lower panels) and western blot analyses (a, right panel) show the amounts of GST fusion proteins and PRK1 used. Western blots were decorated with the indicated antibodies. Nucleosomes purified from LNCaP cells cultivated in the presence or absence of R1881 for 30 min were analysed in western blot (c, right panel). For ChIP (d, e), LNCaP cells were cultivated in the presence or absence of the androgen receptor agonist R1881, transfected with either siRNA (d) or treated with or without Ro318220 (e) as indicated, and subjected to ChIP with the indicated antibodies. The precipitated chromatin was amplified by PCR using primers flanking AREs in the promoter region of the PSA and KLK2 genes. Western blot analysis (d, right panel) verified the specific siRNA-mediated knockdown of PRK1.

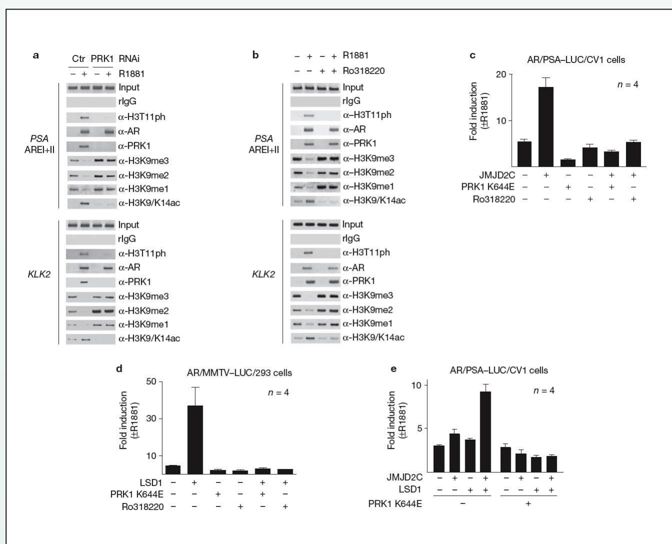

Figure 3 - PRK1 controls modifications of histone H3 and androgen receptor-dependent gene expression. For ChIP (a, b) and transient transfections (c–e), cells were cultivated in the presence or absence of the androgen receptor agonist R1881 and the inhibitor Ro318220 (b–d), or transfected with siRNA (a). ChIP analyses were performed with the indicated antibodies. The precipitated chromatin was amplified by PCR using primers flanking AREs in the promoter region of the PSA and KLK2 genes. For transient transfections, CV1 (c, e) or 293 (d) cells were co-transfected with androgen receptor expression plasmid and androgen receptor-dependent reporters. Bars represent mean + s.d. (n = 4).

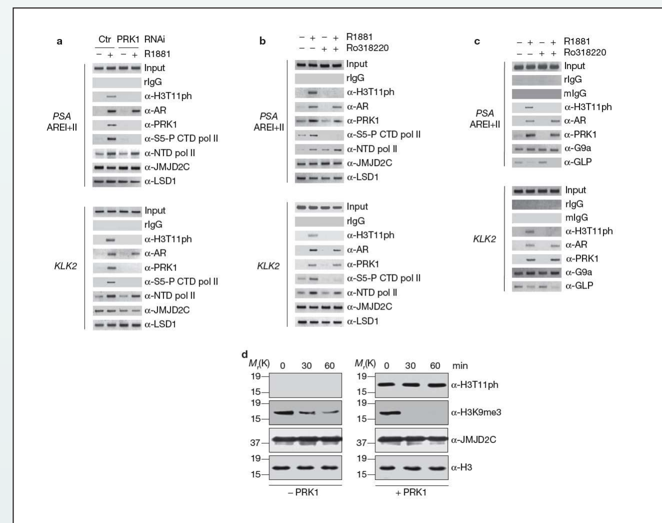

Figure 4 - PRK1 controls transition to the initiation complex and accelerates demethylation by JMJD2C. For ChIP (a–c), cells were cultivated in the presence or absence of the androgen receptor agonist R1881 and the inhibitor Ro318220 (b, c), or transfected with siRNA (a). ChIP analyses were performed with the indicated antibodies. The precipitated chromatin was amplified by PCR using primers flanking AREs in the promoter region of the PSA and KLK2 genes. Untreated nucleosomes (d, left panel) and nucleosomes phosphorylated at H3T11 in vitro (d, right panel) were incubated with 10 mug bacterially expressed and purified His-JMJD2C for the indicated times. Western blots were decorated with the indicated antibodies.

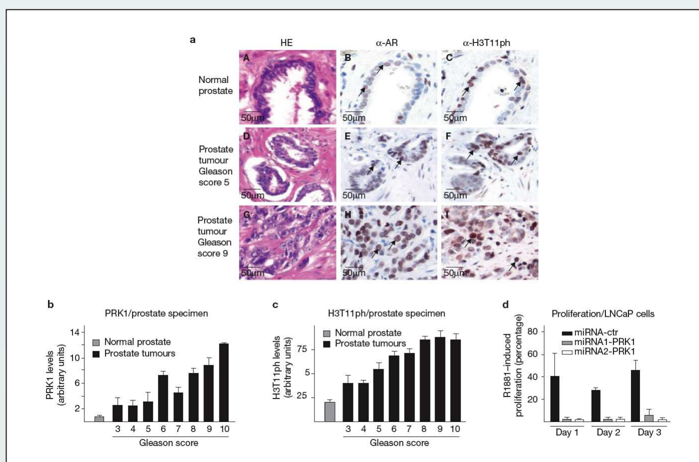

Figure 5 - PRK1 and H3T11ph levels positively correlate with the malignancy of prostate cancer and control proliferation of tumour cells. (a) Immunohistochemical staining of androgen receptor and H3T11ph in human normal and tumour prostate. Androgen receptor (B, E, H) and H3T11ph (C, F, I) immunoreactivity is detected in the secretory epithelium of normal prostate (B, C, arrows) and prostate carcinoma cells (E, F, H, I, arrows). Haematoxylineosin (HE)-stained sections are shown in a, d and g. All sections were taken from the same radical prostatectomy specimen (original magnification, times250). (b, c) The correlation of elevated PRK1 and H3T11ph levels with high Gleason score in a panel of 111 human prostate carcinomas is highly significant: r = 0.499, P < 0.001 (b) and r = 0.450, P < 0.001 (c). Normal prostate specimens (n = 20) are included as a control. (d) In LNCaP cells, miRNA-mediated PRK1 knockdown severely reduces R1881-induced cell proliferation. Bars represent mean + s.d. (n = 5).

Conclusion:

1) **PRK1 phosphorylates histone H3 at threonine 11 (H3T11)**
2) **PRK1 controls AR-dependent transcription**
3) **Levels of PRK1 and phosphorylated H3T11 correlate with Gleason scores of prostate carcinomas**
4) **PRK1 is a potential target to block prostate tumour growth**

Metzger *et al.*, Nat. Cell Biol. 10, (2008)