ATR kinase function is modulated by the proto-oncogene PBF

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Background

Tumourigenesis is a multi-step process necessitating oncogene activation and tumour suppressor gene inactivation[1]. PBF is a proto-oncogene found to be overexpressed in various endocrine cancers including breast and colon cancers[2].

PBF overexpression has shown through *in vivo* experiments to induce tumour formation[3]. Stratford *et al.* showed through *in vitro* experiments that when PTTG and PBF were both overexpressed, cell transformation occurred[3].

Recently, it has been demonstrated that PBF can promote genetic instability by repressing p53 transactivational activity, and reducing p53 stability [4].

The major regulators of the DNA damage response (DDR) are the phosphoinositide-3-kinase related protein kinases (PIKKs) which include ATR and ATM[5]. Together ATM and ATR target an overlapping set of substrates that promote cell cycle arrest, DNA repair, and apoptosis[5].

To further our understanding of the molecular basis of PBF induced genomic instability in greater detail, we investigated whether PBF overexpression affects the DDR regulated by ATM and ATR kinase following ionising or UV radiation. Given the importance of DDR proteins in cell cycle checkpoint control, we also investigated whether overexpression of PBF affects ATM and ATR in cell cycle checkpoint regulation.

Aim

To investigate the molecular mechanism in PBF induced genetic instability and underlying tumourigenesis

Methods

HeLa cells were transfected with either HA-PBF or HA-vector (positive control) before being irradiated with 20 J/m^2 of UV. Cells were harvested and prepared for Western blotting to analyse the protein expression of various proteins involved in the ATR DNA damage response pathway using phospho-specific antibodies.

Different anti-PBF or anti-HA PBF antibodies were used to immunoprecipitate PBF from HeLa cell lysate. Bound ATR proteins were detected by Western blot. Reciprocal co-immunoprecipitation was used to detect any PBF proteins bound to ATR.

To analyse the G2/M checkpoint, all HeLa cells were fixed with 70% (v/v) ice-cold ethanol and cells in mitosis were quantified as the number of phosphor-histone H3 Ser10 (pH3) positive cells by FACS analysis.

PBF and the ATR DNA damage pathway

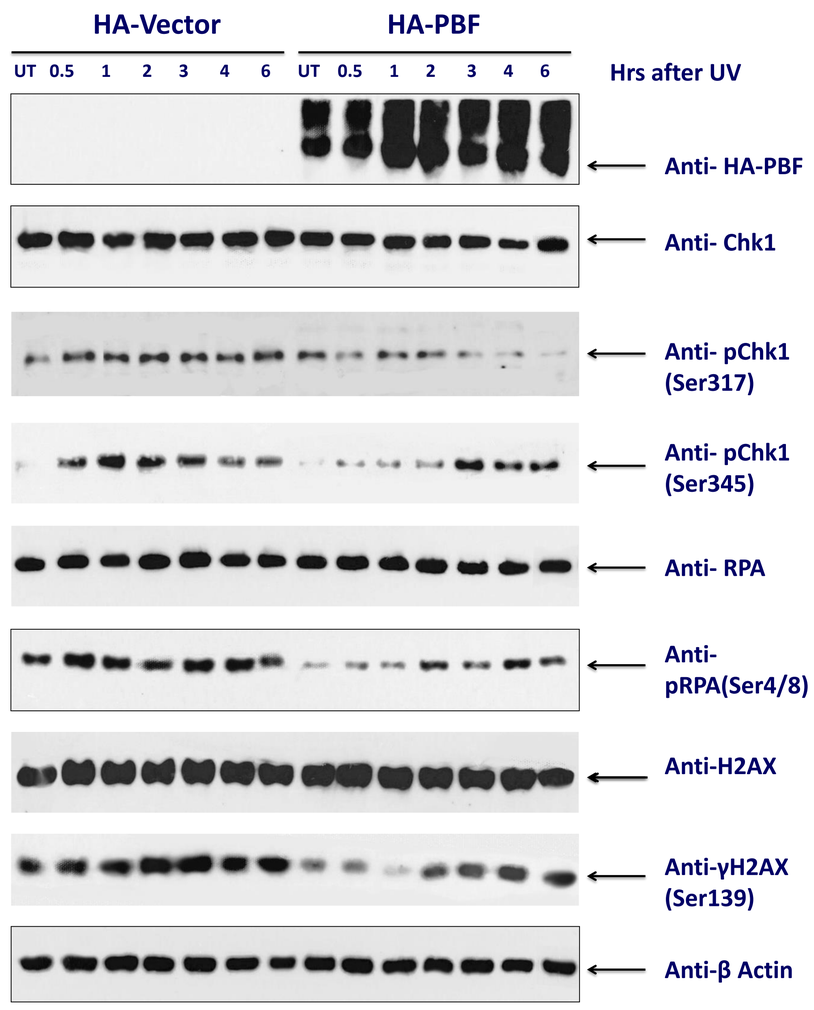

Figure 1. Transient overexpression of PBF suppresses the ATR DNA damage response to UV irradiation. HeLa cells were transfected with either HA-PBF or HA-vector (positive control) before being irradiated with 20 J/m^2 of UV. In cells where PBF was overexpressed, UV treatment activated the ATR DDR pathway as γH2AX, pChk1 and pRPA were all detected. However, PBF overexpression was shown to attenuate considerably the ATR-mediated phosphorylation of Chk1, RPA32, and possibly also H2AX, after UV treatment, relative to UV-treated, HA-vector only controls

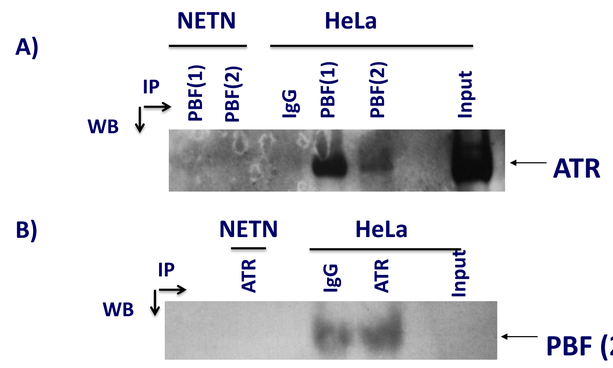

Figure 2 Endogenous PBF binds to ATR in vitro.
(A) Co-immunoprecipitation (co-IP) assays shows that endogenous PBF binds to endogenous ATR. The binding was highly reproducible (n=3). The PBF-ATR interaction was detected specifically in the test samples and not in the negative controls. This was confirmed using reciprocal co-IP assay shown in (B).

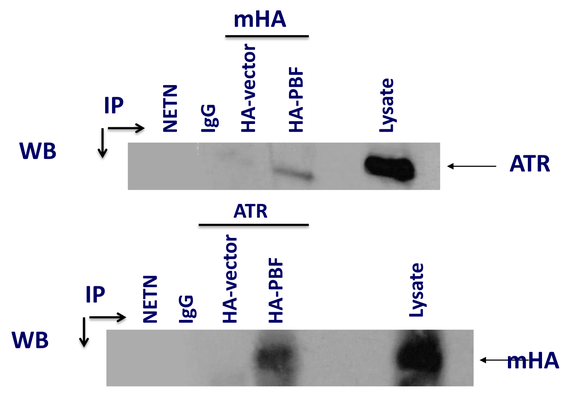

Figure 3 Transfected HA-tagged PBF binds ATR in reciprocal Co-IP assays.
Co-IP assays show exogenous PBF binding specifically to ATR *in vivo.* It is a novel interacting partner of ATR in HeLa cells.

PBF overexpression and the G2/M checkpoint

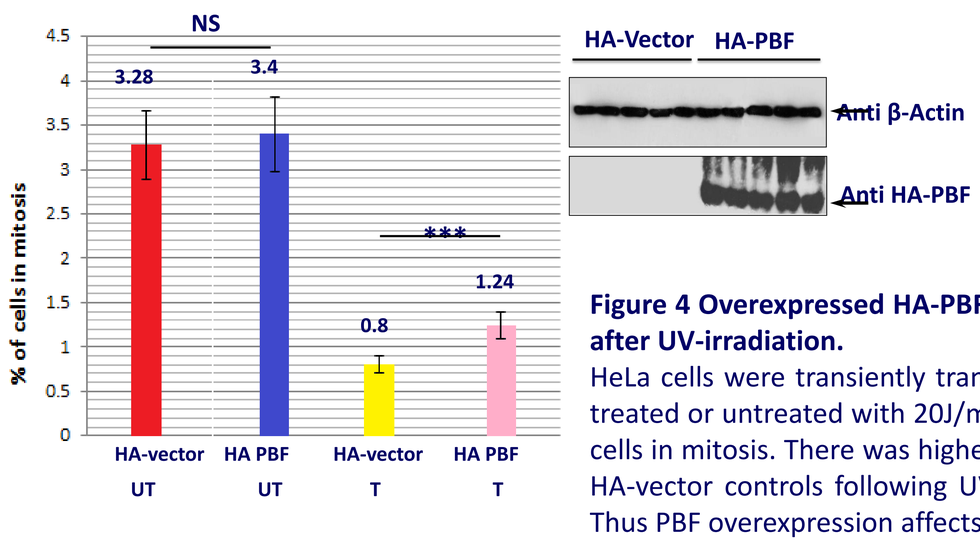

Figure 4 Overexpressed HA-PBF is associated with a higher percentage of cells in mitosis after UV-irradiation.
HeLa cells were transiently transfected with HA-PBF or HA-vector alone. They were then treated or untreated with 20J/m^2 of UV. FACS analysis was used to quantify the number of cells in mitosis. There was higher number of cells in mitosis for HA-PBF samples relative to HA-vector controls following UV-treatment and this was significant at the 0.001% level. Thus PBF overexpression affects the integrity of the G2/M checkpoint.

Conclusions

1. PBF is physiologically important following DNA damage.
2. PBF overexpression reduces the activation of RPA and Chk1 following UV treatment. It also dysregulates the G2/M checkpoint and significantly increases the number of cells entering mitosis in the presence of DNA damage. With the lethal consequences of Chk1 and RPA dysfunction in repair and cell-cycle regulation, PBF overexpression could lead to catastrophic consequences with cell s developing mutagenic potential, genomic instability and ultimately cancer.
3. Both endogenous and exogenous PBF specifically bind ATR *in vivo*. Under normal circumstances PBF may function as a negative regulator or mediator of ATR's ability to phosphorylate and activate ATR substrates. However, when PBF becomes overexpressed, the relationship becomes deleterious and it disrupts the normal DDR.
4. PBF overexpression may therefore confer a survival advantage and proliferative advantage despite DNA damage – facilitating neoplastic growth and tumourigenesis..

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