664 KLF4 suppresses estrogen-dependent breast cancer growth by inhibiting the transcriptional activity of ER alpha

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Background: Breast cancer is typically a hormone-dependent tumour, in which exposure to estrogen increases breast cancer incidence and proliferation via estrogen receptor a (ERα). In this study, we screened negative regulator of breast cancer growth and focused on Kruppel-like factor 4 (KLF4/GKLF/EZF). KLF4 encodes a transcription factor that is associated with tumour suppression. Several lines of evidence indicate that KLF4 is an important regulator of cell proliferation. It was also shown that KLF4 is transcriptionally activated following DNA damage in a p53-dependent manner. Here, we show a novel molecular network between p53, KLF4 and ERα in breast cancer.

Material and Methods: To determine the association of KLF4 with tumour progression, we compared the gene expression profiles in breast cancer using Oncomine database. Subsequently, we performed functional analysis of KLF4 using MTT assay, QRTPCR, reporter assay, Co-immunoprecipitation assay and ChIP assay in MCF-7 cells.

Results: We performed an extensive analysis of the Oncomine database and showed that *KLF4* expression was associated with breast cancer tumourigenesis. Analysis of the database also showed a correlation between *KLF4* expression and ERα-positive breast cancers. *In vitro* experiments using the ERα-positive breast cancer cell line MCF-7 revealed that estrogen-dependent cell growth was significantly enhanced by knockdown of *KLF4*. Co-immunoprecipitation experiments revealed that KLF4 binds to the DNA-binding region of ERα and inhibits the binding of ERα to estrogen response elements in promoter regions to reduce the transcription of ERα target genes. We also showed that activation of p53 decreased ERa transcriptional activity by elevating *KLF4* expression.

Conclusion: Our study uncovered a novel molecular network that involves p53, KLF4 and ERα. Our data indicated that p53 up regulate KLF4, and KLF4 suppresses ERα signaling and estrogen dependent breast cancer proliferation. In future, if we could stimulate KLF4, it will be effective therapies for breast cancer.

665 Estrogen receptor ligands regulate prostate tumourigenesis via ER beta/KLF5 pathway

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Background: Prostate cancer is one of the most prevalent malignancies worldwide, resulting in significant mortality among males. Initially, many patients with prostate cancer are treated using androgen ablation therapy. Most neoplasms, however, will eventually become hormone insensitive. Recent clinical studies suggest that antiestrogens inhibit the development of androgen-insensitive prostate cancer, although the underlying mechanism has not been elucidated.

Material and Methods: Anchorage-dependent and -independent proliferation of prostate cancer cells was analyzed by MTT assay and soft agar colony formation assay. DU145 or PC-3 xenograft tumours were grown in BALB/cA-nu mice. Subcutaneously implanted time-release pellets or injection of agents were used for drug delivery. Western blot analysis was used to measure protein expression and quantitative real-time PCR was used to measure mRNA levels. The apoptosis of prostate cancer cells was determined by TUNEL assay.

Results: *In vitro* experiments showed that ER ligands did not affect on anchorage-dependent proliferation of androgen-insensitive prostate cancer cells (DU145 and PC-3). In contrast, anchorage-independent growth was promoted by 17β-estradiol (E2) treatment and suppressed by ER antagonist ICI 182,780 (ICI). Moreover, *in vivo* experiments showed that ICI inhibits prostate tumourigenesis, whereas E2 enhances prostate tumour formation through ERβ. To reveal this mechanism, we screened ERβ-interacting protein and identified Kruppel-like factor 5 (KLF5). Knockdown of KLF5 expression in prostate cancer cells increased tumourigenesis and abolished the effects of ER ligands. In the presence of ICI, ERβ bound to the promoter of FOXO1 through KLF5, enhancing transcription in a manner dependent on CBP recruitment. Elevated FOXO1 levels increased apoptosis in prostate cancer cells and suppressed tumourigenesis. In contrast, estrogen induced the formation of complexes containing ERβ, KLF5, and the ubiquitin ligase WWP1, resulting in the ubiquitination and degradation of KLF5.

Conclusions: Our results demonstrate that prostate tumourigenesis is suppressed by ICI and enhanced by E2. These effects result from ERβ-mediated regulation of the transcriptional activity of KLF5. In addition, we identified FOXO1 as a critical KLF5 target gene that induces cell apoptosis and suppresses prostate tumourigenesis.

666 Analysis of microRNAs associated with aggressive subtype of chronic lymphocytic leukaemia harbouring inactivated p53

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Background: In chronic lymphocytic leukaemia (CLL), 15% of patients harbour deletion and/or mutation of tumour suppressor gene *TP53*. The p53 inactivation is strongly associated with aggressive course of the disease and poor response to therapy. We have described a group of three cancer-associated microRNAs (miR-34a, miR-29c and miR-17−5p) differently expressed between patients with wild-type (wt) and inactivated p53 (Mraz et al., Leukemia 2009). The basal level of miR-34a, the direct transcriptional target of p53, is high in CLL cells harbouring wt p53 but low in p53-mutated CLL cells and also in normal B-cells. The contribution of miR-34a, miR-29c and miR-17−5p to CLL aggressiveness and chemoresistance and their role in neoplastic transformation of B-cells remains unclear.

Materials and Methods: B-lymphocytes of CLL patients and healthy donors were separated by negative selection (MACS or RossetteSep; B-cell purity >95%). The expression of miRNAs and p53 target genes was analyzed using Real-time PCR (TaqMan MicroRNA Assay, Applied Biosystems). p53 status was routinely assessed by I-FISH and FASAY in all samples.

Results: (1) We have reported that CLL patients may develop novel TP53 mutations during therapy (Malcikova et al., Blood 2010). In such patient, we have analyzed the expression changes of the three miRNAs previously shown to be influenced by p53 and detected ~6.6-fold decrease in miR-34a expression. This result confirms previously published data that miR34a expression is dependent on p53. The levels of miR-17−5p and miR-29c remained unchanged.

(2) To assess the direct changes of these microRNA expression during cytotoxic response, we performed *in vitro* treatment of malignant and non-malignant B-lymphocytes *in vitro* by fludarabine or doxorubicine. In non-malignant B-cells, we observed 4−6 fold increase in miR-34a levels. However, the miR-34a level was only slightly increased (up to 1.5 fold) in primary cultures from previously untreated CLL patients. The p53 functionality was confirmed by monitoring an induction of PUMA and p21 genes.

(3) To confirm the *in vitro* data, the miRNA-34a, 29c and 17−5p changes during chemotherapy *in vivo* are currently analysed and the results will be were correlated to p53 target genes induction.

Conclusions: Our data support the hypothesis that miR-34a participates in p53-dependent cytotoxic response. However, we suppose that the role of miR34a differs in normal and malignant B-lymphocytes.

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667 APC/C plays a role in the acute response to protein-damaging stress

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The heat shock response promotes cell survival in response to protein-damaging stress. Heat shock transcription factors (HSFs) are a family of transcription factors that are activated by stress and induce increased expression of heat shock proteins, that function as molecular chaperones maintaining protein homeostasis. The regulation of HSF activity is crucial, and HSF1 is mainly regulated through posttranslational modifications, whereas different stresses affect the levels of HSF2 by a so far unknown mechanism. The ubiquitin E3-ligase anaphase-promoting complex/cyclosome (APC/C) drives degradation of mitotic regulators in cycling cells by associating with the coactivators Cdc20 and Cdh1. Although a plethora of APC/C substrates have been identified, only a few transcriptional regulators are described as direct targets of APC/C-dependent ubiquitination.

In vivo and in vitro ubiquitination assays were used to show that HSF2 is ubiquitinated in a APC/C-dependent manner. Interactions between HSF2 and the APC/C complex were studied using co-immunoprecipitation studies. Chromatin immunoprecipitation assays were used to study recruitment of HSF2, Cdc20 and proteasome to the *Hsp70* promoter.

Here we demonstrate that APC/C^{Cdc20} mediates ubiquitination and degradation of HSF2, a transcription factor that binds to the *Hsp70* promoter. The interaction between HSF2 and the APC/C subunits Cdc27 and Cdc20 are enhanced by moderate heat stress and the degradation of HSF2 is induced during the acute phase of the heat shock response, leading to clearance of HSF2 from the *Hsp70* promoter. Remarkably, Cdc20 and the proteasome 20S core α2 subunit are recruited to the *Hsp70* promoter in a heat shock-inducible manner, indicating destruction of the promoter-bound HSF2.

Our results provide the first evidence for a participation of APC/C^{Cdc20} in the acute response to protein-damaging stress, expanding the role of