Detection of protein-protein interactions of the corepressor Alien

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Introduction

Alien was identified as a corepressor of some nuclear hormone receptors e.g. thyroid hormone receptor and vitamin D3 receptor. Additionally it was shown to regulate cell cycle, DNA repair and apoptosis. In preliminary studies we found Alien as an interaction partner of E2F1. To get further insight in the cellular functions of Alien we searched for new interaction partners.

Material and Methods

All protein interaction studies presented here were carried out with endogenously expressed proteins to avoid false positive or negative results.
Alien was immunoprecipitated from U2OS cells with an peptide antibody (PepAK2) using Interaction Discovery Mapping (IDM) beads. The antigen and its interaction partners were eluated and analyzed on a SELDI-MS (surface enhanced laser desorption/ionisation mass spectrometer). Proteins were separated on a SDS gel and identified by tryptic digestion. Afterwards results were approved with coimmunoprecipitation with protein A-agarose beads with specific antibodyies against the possible interaction partners.
To investigate the functional relevance of the Alien E2F1 interaction C33A cells were cotransfected with Alien and an E2F1 promotor luciferase reporter. Inhibition of the transcription was measured by a luciferase assay.

Results

We found several new interaction partners of Alien, including at least six members of the E2F family, Retinoblastoma, p107, CDK2 (Fig 1-3) as well as Nucleophosmin, VDRIP, CRSP3, TRIP11 and ERCC3 (data not shown). These proteins have functions in regulation of transcription, DNA repair and cell cycle. Especially proteins that mediate G1-S transition were found to bind to Alien.
Furthermore we investigated what influence Alien has on E2F1 dependent transcription. C33A cells were transfected with Alien and an E2F1 promotor construct. The transcription of the E2F1 promotor luciferase reporter was significantly decreased by Alien compared to the control without overexpressed Alien.

Conclusion

In this study we found that Alien binds to several regulators of the G1-S transition of cell cycle. A functional analyses of these interactions shows an inhibition of E2F1 transcriptional activity. In conclusion, Alien seems to have a great impact of the G1-S transition by regulating transcription as well as degradation of cell cycle associated proteins.

Figures

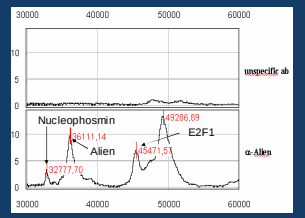

Figure 1:
SELDI spectra of an IP with PepAk2: Peaks with masses similar to E2F1 and Nucleophosmin are shown

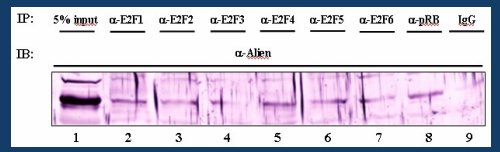

Figure 2: Alien interacts with several members of the E2F family

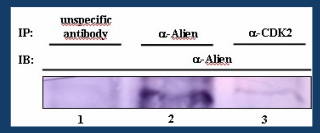

Figure 3: CDK2 interacts with Alien

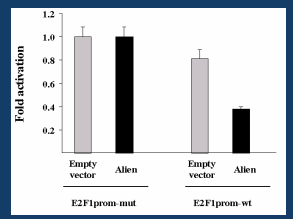

Figure 4:
Influence of overexpression of Alien on E2F1-dependent transcription. Luciferase units were normalised with the β-galactosidase activity of the co-transfected pCMV-lacZ reporter plasmid