RAI2 controls polycomb-mediated repression of CDKN1A by its interaction with CtBP1

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Background & objectives

Elevated levels of RAI2 protein in primary tumors are associated with early biochemical relapse of prostate cancer patients. On the molecular level, RAI2 interacts with CtBP1 as a transcriptional co-repressor via a non-consensus tandem ALDLS-motif in hormone-dependent cancer cells. In this study, we aim to investigate the cell-biological relevance of this molecular interaction in prostate cancer cells using the *CDKN1A* gene as an example of a transcriptional target of CtBP-mediated gene regulation.

Methods

To analyze a molecular relation between the RAI2/CtBP1 interaction and repressor of the *CDKN1A* gene, we first applied a transactivation assay in 293T cells, transiently transfected with CtBP1 and different RAI2 protein variants. Next, we used VCaP cells depleted of RAI2 as a model system to analyze gene and protein expression of RAI2, CtBPs and p21 under different conditions.

Results

The gene reporter assay revealed that combined overexpression of RAI2 and CtBP1 reliefs the repression of the proximal *CDKN1A*-promotor and that RAI2 with an intact ALDLS tandem motif is required for this process. RAI2 depletion in VCaP cells resulted in a significant reduction of both CtBP1 and CtBP2 and almost complete abolishment of p21 protein levels, which is accompanied by reduced interaction of RAI2 with CtBP1 in nuclei. In parental VCaP cells, genotoxic stress significantly induced *CDKNA1* gene expression and p21 protein concentration. In RAI2-depleted VCaP cells we did not observe any of these effects. RAI2 together with CtBP1 appeared as definite foci in the nuclei of VCaP cells that are co-localized with key factors of polycomb 1/2. ChIP analysis revealed that RAI2-depletion significantly induced binding of CtBPs to the *CDKN1A* promoter. In contrast, the binding of LCoR to chromatin is significantly reduced in RAI2-depleted cells, whereas a significant increase in H3K27me3 was observed.

Conclusion

In summary, we found that interruption of the interaction of RAI2 with CtBP1 is leading to increased chromatin binding of CtBP1 and trimethylation of H3K27 of the *CDKN1A* promotor, which is associated with repression of *CDKN1A* gene expression. We conclude that the molecular interaction of RAI2 with CtBP1 is a new molecular mechanism of corepression by modulating the histone-modifications of the target gene.