CK1α INACTIVATION IN MULTIPLE MYELOMA EMPOWERS LENALIDOMIDE INDUCED CYTOTOXICITY AND CELL CYCLE ARREST

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INTRODUCTION

Multiple myeloma (MM) is the second most frequent hematological malignancy. It is characterized by the progressive accumulation of plasma cells in the bone marrow (BM), which provides a favourable microenvironmental *niche* for uncontrolled growth, escape from apoptosis and genetic selection of increasingly biologically aggressive subclones.
The introduction of immunomodulatory drugs, such as lenalidomide (Lena) and pomalidomide, has improved both quality of life and prognosis of patients, which however remain still poor. Novel therapeutic targets are thus urgently needed.
Recently it was demonstrated that in myelodysplastic syndromes and in acute myeloid leukemia, lenalidomide treatment induced a Cereblon-mediated ubiquitination and degradation of protein kinase CK1α [1].
CK1α is a serine/threonine kinase essential for the function of signaling pathways that could be involved in MM pathobiology: the canonical Wnt/β-catenin cascade, Hedgehog , NF-κB pathways and p53-driven response (here by stimulating the binding to Mdm2 and p53 inhibition). Recent evidence indicates that protein kinase CK1α may play a key role in myeloma cell growth and its inactivation determines MM cell death [2].
Therefore, CK1α could be a potential candidate for mediating the cytotoxic /cytostatic effects of lenalidomide also in MM. Here, we sought to investigate the therapeutic potential of the combination of lenalidomide and CK1α inactivation on malignant plasma cells. Inhibition of CK1α in association with lenalidomide may represent an original molecular target approach for MM.

OBJECTIVES

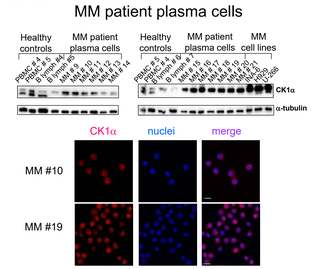
We have analyzed CK1α expression and its contribution to MM survival. We investigated the therapeutic potential of the combination of lenalidomide and CK1α inactivation studying whether lenalidomide could cause CK1α degradation and whether CK1α could take part in lenalidomide-induced MM cell apoptosis and cell cycle arrest. We next studied whether blocking CK1α could influence pro-survival signalling pathways, like β-catenin and AKT accounting for resistance to lenalidomide.

METHODS

MM cell lines: H929, U-266, (ATCC, USA), INA-6 (gift from Dr. M. Gramatzki, University of Kiel, Kiel, Germany)and SaMMi (a patient derived MM cell line, newly generated in our laboratory).

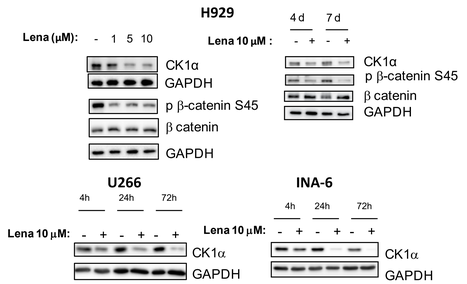
- A model of BM microenvironment was established colturing MM cells with HS-5 stromal cells.
- RNA interference for CK1α was obtained through electroporation of CK1α directed double strand oligonucleotides (AMAXA system). CK1 inhibition was obtained with D4476 (abcam).
- Apoptosis and cell cycle were investigated with AnnexinV/PI and Ki-67/PI stainings and cytofluorimetric analysis. Lenalidomide- and CK1α-dependent signalling events were analyzed by WB.

RESULTS



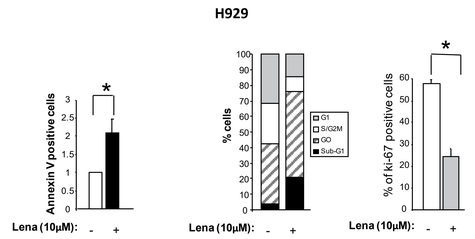
1. *CK1α is highly expressed in MM cells*

CK1α expression and localization in MM (patient derived CD138+ plasmacells and MM cell lines U-266, INA-6 and H929) and control cells (Peripheral Blood Mononucleated Cells (PBMC) or B lymphocytes from healthy donors).



2. *Lenalidomide causes cytotoxic and cytostatic effects on MM cells*

H929 cells were treated with Lena 10 μM for 7 days. The histogram on the left panel shows Annexin V + cells, the histogram in the middle shows the average of 3 independent experiments of cell cycle analysis, while the bar graph on the right side shows the mean ±SEM of Ki-67+ cells. * indicates p<0.05.

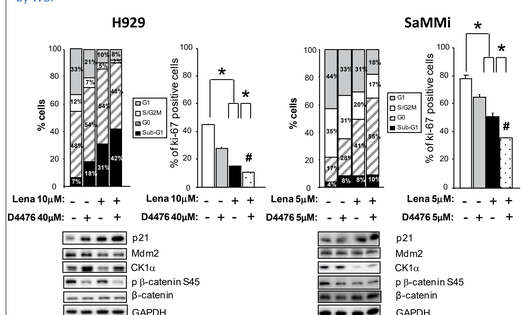


3. *Lenalidomide determines CK1α protein reduction in a dose and time dependent manner, with a decrease of its kinase activity*

H929 cells were treated with Lena at different concentrations (1, 5, 10 μM) for 7 days (left panel) or with Lena 10 μM for 4 (4d) and 7 days (7d). U-266 and INA-6 were treated with Lena 10 μM for 4h, 24h and 72h. CK1α, phosphorylated β-catenin on Ser 45 (p β-catenin S45) and total β-catenin was evaluated by WB.

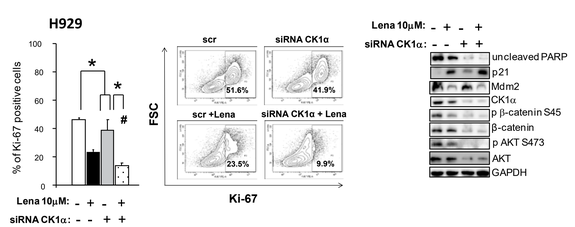
4. *CK1 inhibition empowers lenalidomide induced apoptosis*

MM cells were cultured for 7 days (H929) or 3 days (SaMMi). Lena was added at t=0h and D4476 was added 48h before harvesting. Annexin V /PI staining and FACS analysis of H929, (left panel), SaMMi grown alone (middle panel) or in cocolture with HS-5 (SaMMi/HS-5, right panel). * indicates p<0.05. ● and # indicates p<0.05 between sample treated with D4476 alone and Lena together with D4476.



5. *CK1 inhibition empowers lenadomide induced cell cycle arrest*

MM cells were cultured for 7 days (H929) or 3 days (SaMMi). Lena was added at t=0h and D4476 was added 48h before harvesting. Cell cycle analysis with Ki-67/PI staining and Ki-67 in H929 (left) and SaMMi cells (right). * indicates p < 0.05, # indicates p<0.05 between sample treated with D4476 alone and Lena together with D4476. Expression of p21, Mdm2, CK1α, p β-catenin S45 and total β-catenin proteins (bottom panels).



6. *CK1α silencing cooperates with lenalidomide in inducing cell cycle arrest and modulates β-catenin and AKT signalling*

Ki-67 positive H929 cells transfected with CK1α directed siRNAs and treated for 3 days with Lena (average of five independent experiments). * indicates p < 0.05. # indicates p < 0.05 between samples treated with the combination of CK1α silencing and Lena and Lena only treated cells. The middle panel shows a representative Contour Plot. Right panel: signaling proteins evaluated by WB.

CONCLUSIONS

- **Lenalidomide determines CK1α protein degradation with a subsequent reduction of its kinase activity**
- **CK1α inactivation empowers lenalidomide induced cytotoxic/cytostatic effects, changing cell cycle related protein expression (p21, Mdm2)**
- **CK1α modulates β-catenin and AKT survival signalling pathways expression and phosphorylation**
- **In the clinical setting, the combination of CK1α inhibitors and lenalidomide could be useful to increase lenalidomide efficacy.**

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