Chetomin, targeting HIF-1α/p300 complex, exhibits antitumour activity in multiple myeloma

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Background: Multiple myeloma (MM) is an incurable clonal plasma cell malignancy. The constitutive expression of HIF-1α in MM suggests that inhibition of HIF-1α-mediated transcription represents an interesting target in MM.

Methods: As p300 is a crucial co-activator of hypoxia-inducible transcription, disrupting the complex HIF-1α/p300 to target HIF activity appears to be an attractive strategy.

Results: We reported that chetomin, an inhibitor of HIF-1α/p300 interaction, exhibits antitumour activity in human myeloma cell lines and primary MM cells from patients.

Conclusions: Our data suggest that chetomin may be of clinical value in MM and especially for patients characterised by a high EP300/HIF-1α expression and a poor prognosis.

Keywords: Multiple Myeloma; HIF-1 alpha; p300; Chetomin; Targeted therapy
According to these data, disrupting the complex formed by HIF-1α and p300 appeared to be a promising approach to target HIF (Colla et al, 2007; Storti et al, 2013; Reece et al, 2014).

Chetomin, a metabolite complex, produced by several fungi of the genus *Chaetomium*, disrupts the ability of tumours to adapt to hypoxia by blocking the HIF pathway (Kung et al, 2004). Chetomin targets p300, a transcriptional co-activator, by disrupting the structure of its CH1 domain. By this way, chetomin makes impossible the interaction between p300 and HIF-1α, which results, in a mitigation of hypoxia-inducible transcription (Kung et al, 2004; Reece et al, 2014). The aim of this study was to investigate the interest of chetomin as a strategy for MM treatment.

**MATERIALS AND METHODS**

Human myeloma cell lines (HMCL, n = 10) XG-1, XG-2, XG-6, XG-7, XG-11, XG-13, XG-19, RPMI8226, LP1 and SKMM2 were obtained as previously described (Moreaux et al, 2011) or purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany) and American Type Culture Collection (Rockville, MD, USA). Microarray data are deposited in the ArrayExpress public database (accession numbers E-TABM-937 and E-TABM-1088). Human myeloma cell lines were cultured with graded chetomin (Sigma, St Louis, MO, USA) concentrations. Human myeloma cell lines’ cell growth was quantified with a Cell Titer Glo Luminescent Assay (Promega, Madison, WI, USA) and the 50% inhibitory concentration (IC50) was determined using GraphPad Prism software (http://www.graphpad.com/scientific-software/prism/).

Gene expression profiling (GEP) of MM cells (MMCs) were obtained from two independent large patients’ cohorts: the Heidelberg–Montpellier (HM, N = 206) cohort (ArrayExpress public database under accession number E-MTAB-362) (Hose et al, 2011; Moreaux et al, 2012) and the publicly available cohort from University of Arkansas for Medical Sciences (UAMS, Little Rock, AR, USA, cohort treated with total therapy 2, N = 345) (GEO, http://www.ncbi.nlm.nih.gov/geo/, accession number GSE2658). Patients presenting with previously untreated MM (N = 206) at the university hospitals of Heidelberg and Montpellier have been included in the study approved by the ethics committee of Montpellier (DC-2008-417) and Heidelberg after written informed consent in accordance with the Declaration of Helsinki. Clinical parameters and treatment regimens of the MM patients included in the HM cohort were previously described (Moreaux et al, 2012).

Bone marrow of patients presenting with previously untreated MM (N = 6) at the university hospital of Montpellier was obtained after patients’ written informed consent in accordance with the Declaration of Helsinki and agreement of the Montpellier University Hospital Centre for Biological Resources (DC-2008-417). Primary myeloma cells of patients were cultured with or without graded concentrations of chetomin and MMC cytotoxicity evaluated using anti-CD138-phycocerythrin monoclonal antibody (Immunotech, Marseille, France) as described (Moreaux et al, 2012).

**RESULTS**

A combined high HIF-1α and EP300 expression, defined using maxstat R package (Kassambara et al, 2012), in MMCs could predict for shorter overall survival (OS) in two independent cohorts of patients (P = 0.007 in the HM cohort N = 206 and

Figure 1. High HIF1-α and EP300 expression in MMCs could predict for shorter overall and event-free survival (EFS) in two independent cohorts of patients. (A) Patients of the HM cohort (n = 206) were ranked according to increasing EP300 and HIF-1α expression and a maximum difference in OS was obtained using the Maxstat R function. High EP300 and HIF-1α expression is also associated with a shorter EFS in the HM cohort (n = 206). (B) High EP300 and HIF1α expression is associated with a poor prognosis (OS and EFS) in an independent cohort of 345 patients (LR-TT2 cohort).
$P = 0.0001$ in the UAMS-TT2 cohort ($N = 345$) (Figure 1). Low-risk patients accounted for 85.4% with a not reached median OS and high-risk patients for 14.6% with a median OS of 54.9 months in the HM cohort (Figure 1). In the UAMS TT2 cohort, a significant better survival in $EP300^{\text{low}}/HIF-1\alpha^{\text{low}}$ group (median OS not reached) compared with $EP300^{\text{high}}/HIF-1\alpha^{\text{high}}$ group (median OS of 56 months; $P = 0.0001$) was also found (Figure 1). Patients with $EP300^{\text{high}}/HIF-1\alpha^{\text{high}}$ expression have also a median event-free
survival significantly decreased compared with those of the EP300(high)/HIF-1α(low) group (P = 0.004 in the HM cohort and P = 0.0003 in the UAMS TT2 cohort).

Gene set enrichment analysis was performed to compare gene expression profiles of patients myeloma cells with EP300(high)/HIF-1α(high) and EP300(low)/HIF-1α(low) expression. Genes overexpressed in MM molecular subgroups CD2 and LB (low bone disease) were significantly enriched in EP300(low)/HIF-1α(low) group (Supplementary Figure S1A and Supplementary Tables S1 and S2). Patients of the EP300(high)/HIF-1α(high) group were characterised by a significant enrichment of genes related to embryonic stem cells, DNMT1 targets and proliferation (Supplementary Figure S1B and Supplementary Tables S3–S5). According to these data, we investigated the correlation between HIF-1α/EP300 expression and MM plasma cell labelling index or GEP-based growth proliferation index (GPI) (Hose et al, 2011). No significant correlation between HIF-1α/EP300 expression and myeloma cell proliferation or GPI was identified (Supplementary Figures S2A and B).

The effect of chetomin was investigated in 10 different HMCL representative of the patients’ molecular heterogeneity (Moreaux et al, 2011). Chetomin induced a dose-dependent inhibition of cell growth in all investigated HMCL, independently of the their molecular heterogeneity (Moreaux et al, 2011) with a median IC50 of 4.1 nM (range: 2.29–6.89 nM) (Figure 2A). Chetomin treatment induced a significant downregulation of HIF-1α target gene expression (Storti et al, 2013) including VEGF, IL-8 and CLL3 proangiogenic genes (Supplementary Figure S3).

We investigated the effects of chetomin on primary MM cells of patients (n = 6, Supplementary Table S6) co-cultured with their bone marrow microenvironment (Moreaux et al, 2012). After 4 days of treatment, cells were enumerated and the fraction of viable myeloma cells (CD138+), non-myeloma cells (CD138−) and haematopoietic progenitors (CD34−) were determined by flow cytometry (Moreaux et al, 2012). Chetomin induced a dose-dependent toxicity on myeloma cells of patients, with a median IC50 of 1.56 nM without affecting the survival of bone marrow normal cells or CD34+ haematopoietic stem cells (Figure 2B).

Interestingly, EP300(high)/HIF-1α(high) myeloma cells of patient 2 were the most sensitive to low-dose chetomin treatment (Supplementary Table S6 and Supplementary Figure S5).

It was previously demonstrated that HIF-1α suppression enhanced the anti-myeloma activity of melphalan and lenalidomide (Hu et al, 2009; Storti et al, 2013). Low concentration of chetomin (2 nM) significantly enhanced the anti-myeloma activity of lenalidomide and melphalan (Supplementary Figures S4A and B).

**Discussion**

According to our data, targeting HIF-1α/p300 interaction appears to be a potent strategy to target hypoxia pathway in MM. Studies have demonstrated a positive regulation of HIF1-α by MM growth factors including IL6 and IGF-1 (Borsi et al, 2014), a correlation between HIF1-α expression and MYC deregulation (Zhang et al, 2009) and a role of HIF1-α in MM angiogenesis through stimulation of VEGF (Asosingh et al, 2005; Colla et al, 2007). Interestingly, chetomin treatment induced a significant anti-myelomatous activity without toxicity on bone marrow normal cells and haematopoietic progenitors (Figure 2B).

High HIF-1α and EP300 expression are associated with a poor prognostic value in MM (Figure 1). The prognostic value of EP300(high)/HIF-1α(high) expression was compared with usual prognostic factors—ISS, t(4;14), del17p, or published GEP-based risk scores including UAMS-HRS, IFM score, GPI and RS score. Using univariate Cox analysis on HM cohort, all these factors had prognostic value (Supplementary Table S6). When these parameters were compared two by two, EP300(high)/HIF-1α(high) expression tested with β2m, del17p, GPI, HRS, IFM and ISS remained significant (Supplementary Table S6). When tested together, only RS, β2m and t(4;14) kept prognostic value (Supplementary Table S6). EP300(high)/HIF-1α(high) MM patients are characterised by a significant enrichment of genes related to proliferation, stemness and DNMT1 targets. We previously reported genes signatures shared by MM cells and normal stem cells linked with a prognostic value and that might be important in malignant stem cell biology (Kassambara et al, 2012). Furthermore, HIF-1α represents an interesting target to eradicate cancer stem cells in haematological malignancies including lymphoma and acute myeloid leukemia (Wang et al, 2011). Chetomin could be useful to target MM patients with a high myeloma-stem cell score and aggressive disease (Kassambara et al, 2012). Furthermore, chetomin enhances the toxicity of lenalidomide and melphalan on MM cells as previously reported with HIF-1-α depletion (Hu et al, 2009; Storti et al, 2013).

Altogether, these data suggest that chetomin or HIF-1-α/p300 inhibitors may be of clinical value in MM and especially for patients characterised by a high EP300 and HIF-1α expression.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Author Contributions**

EV performed research, bioinformatic studies and participated in the writing of the paper. CG participated in the research. AG and DH participated in clinical data analysis and writing of the paper. BK participated in the research and in the writing of the paper. JM supervised the research, bioinformatic studies and the writing of the paper.

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