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Short communication

ZNF224 is a transcriptional repressor of AXL in chronic myeloid leukemia cells

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ABSTRACT

ZNF224 is a KRAB-zinc finger transcription factor that exerts a key tumor suppressive role in chronic myelogenous leukemia.

In this study, we identify the receptor tyrosine kinase Axl as a novel target of ZNF224 transcriptional repression activity. Axl overexpression is found in many types of cancer and is frequently associated with drug resistance. Interestingly, we also found that sensitivity to imatinib can be partly restored in imatinib-resistant chronic myelogenous leukemia cells by ZNF224 overexpression and the resulting suppression of Axl expression.

These results, in accordance with our previous findings, support the role of ZNF224 in imatinib responsiveness and shed new insights into potential therapeutic use of ZNF224 in imatinib-resistant chronic myelogenous leukemia.

1. Introduction

The KRAB zinc-finger transcription factor ZNF224 plays a pro-apoptotic and antiproliferative role in chronic myelogenous leukemia (CML), acting as a cofactor of Wilms' tumor 1 (WT1) protein and modulating WT1 dependent transcription of apoptotic genes [1,2]. Interestingly, we also observed that ZNF224 expression is negatively regulated by Bcr-Abl fusion protein in CML cell lines and in CML patients and, consistently, inhibition of Bcr-Abl activity by imatinib and second-generation tyrosine kinase inhibitors (TKIs) triggers upregulation of ZNF224 expression [3]. Moreover, we recently demonstrated that ZNF224 is a novel transcriptional repressor of c-Myc oncogene in CML and mediates the imatinib-dependent transcriptional repression on c-Myc. In agreement with the role played by c-myc in imatinib responsiveness [4], we also provided evidence that ZNF224 induction, leading to c-Myc repression could contribute to overcome imatinib resistance [5], that represents a key issue in CML research and challenge in clinical practice.

On the other hand, recent findings reported the overexpression of the receptor tyrosine kinase Axl as a mechanism of resistance to TKIs, independent from Bcr-Abl mutations [6,7].

Axl is a receptor tyrosine kinase of the TAM family and was originally isolated as a transforming gene from patients with chronic myeloid leukemia (CML) [8].

Activation of Axl by growth arrest-specific protein 6 (GAS6) ligand, regulates a number of pro-survival pathways, such as RAS, Pi3K/Akt, and mTOR. Axl is overexpressed in several haematologic and solid malignancies and its overexpression drives a wide range of processes, including epithelial to mesenchymal transition, tumor angiogenesis, resistance to chemotherapeutic and targeted agents [9–13]. However, despite the role of Axl in controlling various malignant properties, the transcription factors involved in the modulation of its expression are still largely unknown.

Axl overexpression has been found in Imatinib-resistant CML cell lines and patients [14]. Consistently, it has been demonstrated that Axl knockdown re-sensitized TKI-resistant CML cells to Imatinib, while Axl overexpression confers a refractory response to Imatinib in CML cells [15].

Therefore, understanding of molecular mechanisms involved in Axl overexpression could lead to the development of new

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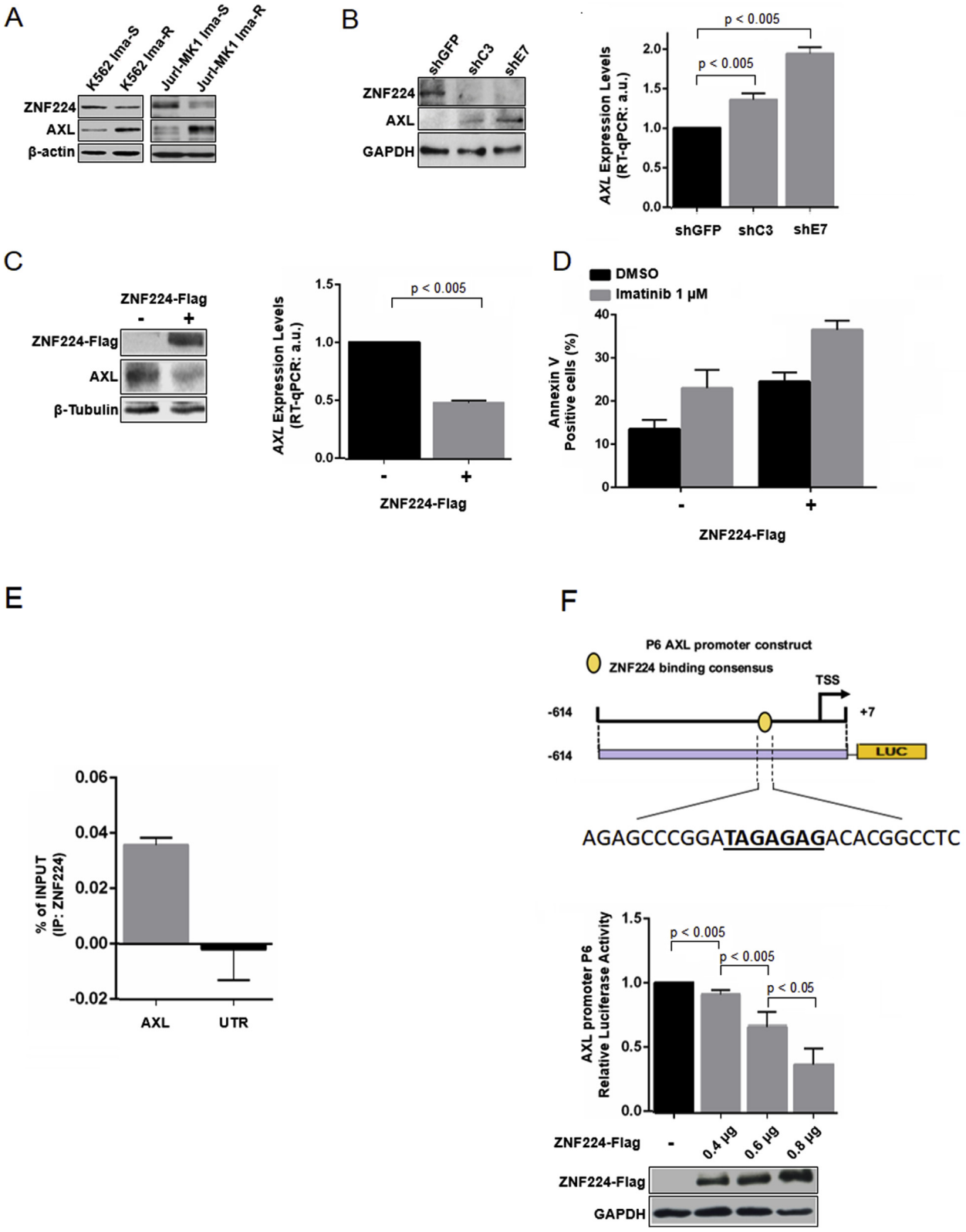

Fig. 1. ZNF224 is a transcriptional repressor of Axl. (A) Western blot analysis of ZNF224 and Axl protein levels in K562 Ima-S and Ima-R, Jurl-MK1 Ima-S and Ima-R. β-actin was used as loading control. (B) Left panel: Western blot analysis of ZNF224 and Axl protein levels in shGFP, shC3 and shE7 cells. GAPDH was used as loading control. Right panel: Axl mRNA levels were measured by RT-qPCR. Error bars represent standard deviations of three independent experiments. (C) Left panel: Western blot analysis of ZNF224-Flag and Axl protein levels in K562 cells transfected with 1,5 μg of ZNF224-Flag expression plasmid or Flag empty vector as control (−). β-tubulin was used as loading control. Right panel: Axl mRNA levels were measured by RT-qPCR. Error bars represent standard deviations of three independent experiments. (D) Apoptosis was evaluated after treatment with 1 μM imatinib or vehicle only (DMSO). Error bars represent standard deviations of two independent experiments. (E) X-ChIP assay performed in HEK293 cells with an anti-ZNF224 antibody. The immunoprecipitated DNA was analyzed by qPCR using primers flanking the putative ZNF224 binding region. An unrelated region was used as negative control (UTR). Data shown are the means ± SD of two independent experiments. (F) Upper panel: Schematic representation of Axl promoter region showing the putative ZNF224 binding