Hypomethylation of IRX1 a poor marker in circulating tumor DNA and drives osteosarcoma metastasis via CXCL14/NF-kB upregulation

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Abstract

Epigenetic abnormalities play a crucial role in osteosarcoma development. However, the epigenetic mechanisms involved in osteosarcoma metastasis remain largely unknown. Here, we analyzed two syngeneic primary human osteosarcoma cell lines with distinct metastatic potentials using methylated DNA immunoprecipitation (MeDIP) and microarray expression analysis to screen for metastasis-promoting genes linked to epigenetic alterations. We identified Iroquois homeobox 1 (IRX1) as a candidate metastasis-promoting gene. IRX1 overexpression was strongly associated with hypomethylation of its own promoter in human osteosarcoma cell lines and clinical osteosarcoma tissues. Furthermore, experimental modulation of IRX1 in osteosarcoma cell lines profoundly altered metastatic activities including migration, invasion, and anoikis resistance in vitro and lung metastasis in vivo. These pro-metastatic effects of IRX1 were mediated by the upregulation of CXCL14/NF-κB signaling. Notably, hypomethylation of IRX1 in circulating tumor DNA in the serum of osteosarcoma patients predicted a poor likelihood of lung metastasis-free survival. This study identified IRX1 as a hypomethylated pro-metastatic gene and suggested that (1) IRX1 hypomethylation could be a new molecular marker for early detection of lung metastasis and (2) epigenetic reversion of IRX1 activation may be beneficial for controlling osteosarcoma metastasis.

The molecular detection of epigenetic changes in cell-free tumor DNA in serum has been highlighted as a potential tool for cancer diagnosis and prognosis. Compared to tumor tissue-based biomarkers, circulating blood biomarkers are easily available and less invasive. It has been demonstrated that epigenetic changes in cell-free tumor DNA can be detected in the serum of cancer patients. To determine whether hypomethylation of IRX1 in serum DNA is a potential

marker for lung metastasis of osteosarcoma, we examined the methylation status of the IRX1 promoter by methylation-specific PCR (MSP) in 67 serum samples collected from primary osteosarcoma patients between August 2010 and September 2012. Representative results of MSP for the IRX1 promoter are shown in Figure 12A. There was no association between IRX1 promoter methylation in the serum DNA and patient age, gender, tumor site or Enneking stage . However, we found that the IRX1 promoter was hypomethylated in 34.2% of serum samples (13 of 38) from patients without metastases, while 62.1% of serum samples (18 of 29) from the metastatic group showed IRX1 promoter hypomethylation (P=0.023, χ^2 test). Kaplan-Meier analysis indicated that patients with hypomethylated IRX1 in their serum exhibited worse lung metastasis-free survival than those with hypermethylation, suggesting that the detection of IRX1 promoter hypomethylation in the serum DNA of osteosarcoma patients could be a potential predictive marker for monitoring lung metastasis in osteosarcoma. Given that aberrant gene methylation is one of the earliest molecular changes that occurs during cancer progression , the detection of IRX1 hypomethylation in serum DNA could be a promising strategy for the early detection of osteosarcoma lung metastasis and might be useful for guiding individual treatment and assessing the early response to chemotherapy.

Lu J, Song G, Tang Q, Zou C, Han F, Zhao Z, Yong B, Yin J, Xu H, Xie X, Kang T, Lam Y, Yang H, Shen J, Wang J*. IRX1 hypomethylation promotes osteosarcoma metastasis via induction of CXCL14/NF-κB signaling. J Clin Invest. 2015;125(5):1839–1856. doi:10.1172/JCI78437. Feature Article，JCI impact.(Published)

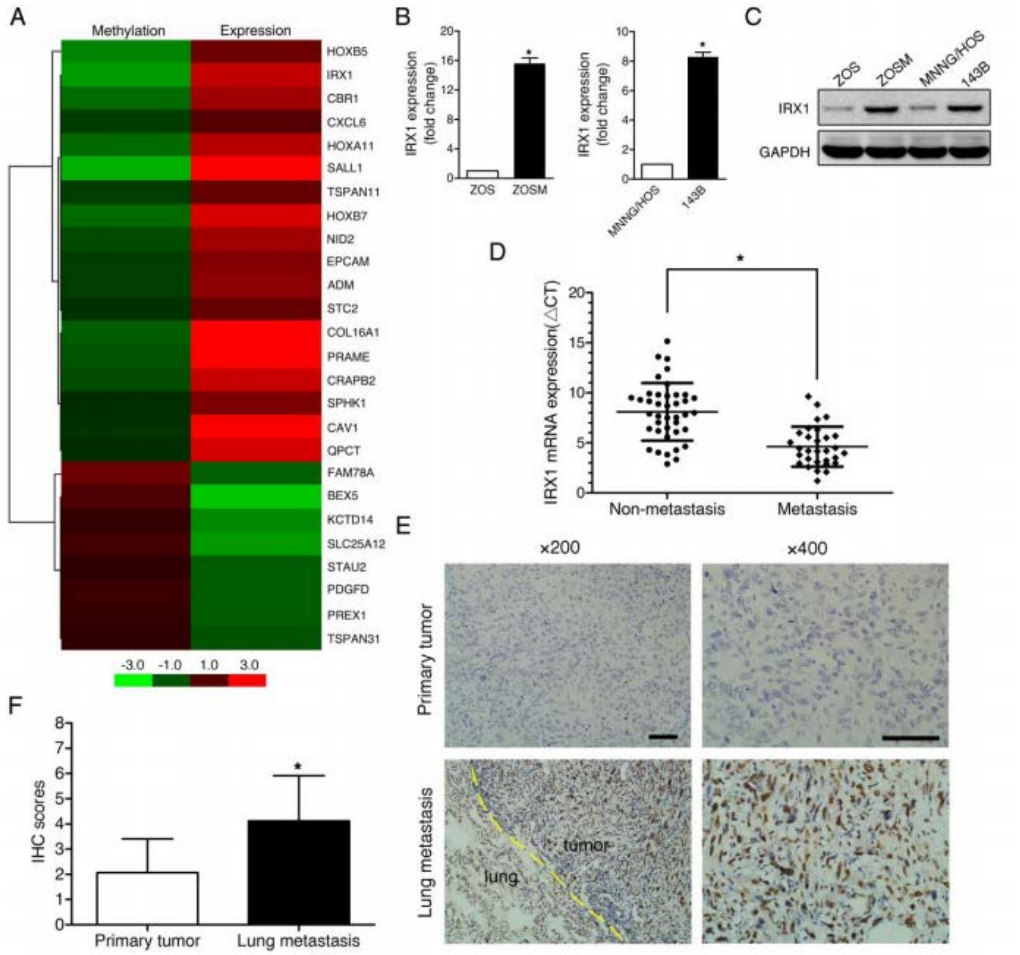

Figure 1

A high-throughput method to screen for epigenetically activated metastasis-driving genes in osteosarcoma. (A) Clustering map of the MeDIP and expression array data obtained from the two primary osteosarcoma cell lines ZOSM and ZOS. (B) Real-time PCR analysis. The values shown are the mean ± SD of 3 separate determinations. (C) Western blot analysis. (D) IRX1 mRNA expression in human osteosarcoma tissue (n=70) was determined by real-time PCR. (E) Immunohistochemical staining of IRX1 in 16 pairs of primary osteosarcoma samples and their corresponding lung metastasis tissues. Scale bars: 100 μm. (F) Statistical analysis (Wilcoxon signed-rank test) showed a significant increase in IRX1 expression in lung metastases relative to the expression in primary osteosarcoma samples. *P<0.05.

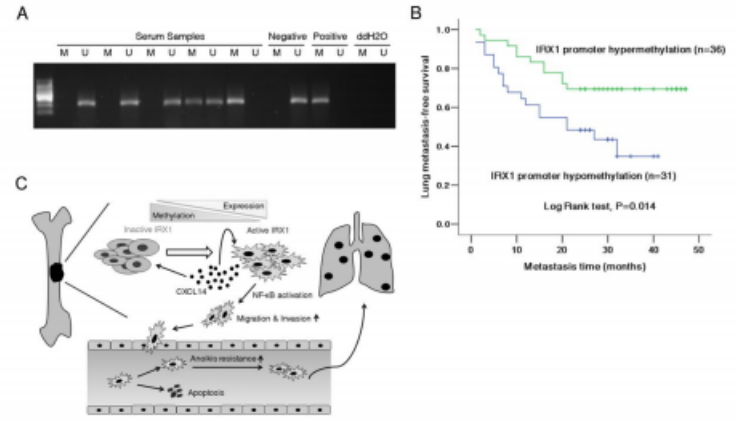

Figure 12

Prognostic relevance of IRX1 promoter methylation in serum DNA from osteosarcoma patients. (A) Methylation-specific PCR analysis of the IRX1 promoter region in the serum DNA of osteosarcoma patients (n=67). M: hypermethylated IRX1; U: unmethylated IRX1. Representative images of serum samples, including negative and positive controls, are shown. (B) Patients with hypomethylation of the IRX1 promoter in their serum DNA had a lower rate of lung metastasis-free survival (log rank test, P=0.014). (C) Potential mechanism by which IRX1 promotes osteosarcoma metastasis. IRX1 is activated by hypomethylation of its own promoter as osteosarcoma progresses. Overexpression of IRX1 leads to increased autocrine of CXCL14 and promotes metastatic behaviors of osteosarcoma cells via NF-κB activation.