INTRODUCTION:
Integrin-linked kinase (ILK) is a serine/threonine protein kinase which activates a range of signaling pathways. ILK is implicated in multiple aspects of tumor progression such as cell survival, invasion and angiogenesis. Moreover, prognostic significance of ILK was shown in melanoma and malignancies of ovary, prostate and stomach. We asked if ILK expression can serve as a marker for disease progression and is associated with tumor progressive effect of angiogenesis, apoptosis and cell invasion in osteosarcoma.

METHODS:
ILK expression was evaluated by immunohistochemistry in 56 pre-chemotherapy surgical specimens obtained from patients who were treated at our hospital. Level of ILK expression was graded according to the extent (grades 0-4) and the intensity (grades 0-3) of staining. The level of ILK expression was considered high when the staining score (extent*intensity) was greater than 7. Correlation between ILK expression and various clinicopathologic variables was analyzed. Survival curves were determined using the Kaplan-Meier method, with the Log-rank test used to evaluate the differences. The Cox proportional hazards model was used for multivariate regression analysis of survival data. To study the role of ILK in multiple aspects of tumor progression, knock-down of ILK by short-interfering RNA (siRNA) transfection was performed in osteosarcoma cell lines (U2-OS and MG63). The efficacy of transfection was assessed with RT-PCR and Western blot analysis. The ILK-siRNA transfected cells were subjected to various experiments and compared with control cells. To investigate the role of ILK in angiogenesis, ELISA for VEGF, a potent pro-angiogenic factor was performed in osteosarcoma cell lines. The role of ILK in cell invasion and apoptosis was studied by Matrigel chamber assay and Annexin-V FACS analysis, respectively. The institutional review board of the hospital approved this study.

RESULTS:
1. ILK is expressed in osteosarcoma. ILK was expressed in 93% (n=52) of specimens by immunohistochemistry. A prostate adenocarcinoma specimen was used as positive control. Level of ILK expression was high in 14 specimens (25%).

2. High ILK expression is related with worse outcome in osteosarcoma. High ILK expression was associated with a worse overall survival (p = 0.004) and event-free survival (p = 0.003). ILK was an independent prognostic factor for overall survival (p = 0.022) but did not reach significance for event-free survival (p = 0.059).

3. siRNA targeting ILK down-regulates ILK expression in osteosarcoma cell lines. Transfection of siRNA into osteosarcoma cell line (U2-OS) resulted in dose-dependent knock-down of ILK as shown by the PCR (upper row) and Western blot (lower row). The most effective silencing of ILK was achieved at 2µM of siRNA and this optimal scheme was used for further experiments.

4. ILK inhibition results in decreased VEGF production in osteosarcoma cells. VEGF concentration in the supernatants of ILK-siRNA transfected osteosarcoma cell lines was down-regulated compared to control cells.

5. ILK inhibition results in increased apoptosis in osteosarcoma cells. Knockdown of ILK by siRNA resulted in increased apoptosis in osteosarcoma cell lines compared to control cells.

6. ILK inhibition reduced the invasiveness of osteosarcoma cells. The invasive ability of ILK-siRNA transfected U2-OS cells decreased compared to control cells.

DISCUSSION:
Our findings suggest that ILK expression may serve as a marker for disease progression and that ILK correlates with angiogenesis, apoptosis and invasion in osteosarcoma.

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