Methylation of the ER-alpha Promoter Is Influenced by its Ligand Estrogen in Osteosarcoma Cells SAOS-2 In Vitro.

Abstract:

The aggressive fast-growing osteosarcoma is the most common primary malignant bone tumor. The relevance of estrogen as a key player in bone metabolism and bone tumor is well-known. At the molecular level, estrogen activates the estrogen receptor α (ERα) as a natural ligand of this receptor. ERα acts as a transcription factor by binding to the "estrogen response element" (ERE) and regulates the expression of a various number of genes. Epigenetic processes, e.g. the methylation of the "cytosine-phosphatidyl-guanine (CpG) islands" can change the transcription of target genes and subsequently the protein expression. As DNA methylation is generally associated with gene transcription repression, up until now little is known about the ERα methylation in osteosarcoma cells. The aim of the present pilot study was to evaluate the methylation status of ERα in osteosarcoma cells SAOS-2 and MG 63 after stimulation with estrogen. SAOS-2 and MG 63 cells were cultured in DMEM. After treatment with 10 nmol estrogen (E2) for 24 h, the expression of ERα was detected by immunocytochemistry (ICC). As controls we used untreated cells. Staining was evaluated semi-quantitatively by the immunoreactive score of Remmele and Stegner (IRS). To determine mRNA gene expression, extracted RNA was transcribed into c-DNA and a quantitative real-time-PCR
(qRT-PCR) was carried out. The semi quantitative evaluation of the ER? mRNA was based on the 2(-ΔΔct) method using untreated cells as reference control. One microgram of each extracted genomic DNA sample was converted with bisulfite and a real-time methylation-specific PCR (rt-MSP) was performed. The estrogen-stimulated SAOS-2 cells showed a significant increase of ER? expression. A 7-fold up-regulation of ER? mRNA confirmed the results of immunocytochemistry. Methylation of the ER? promoter was not detected in treated cells. In contrast, we identified methylation of the ER? promoters in untreated cells. The staining of MG 63 cells showed a weak gain of ER? expression in the stimulated cells, as well as a weak increase of the ER-? mRNA (2-fold). Methylation of the ER? promoters was not detectable in either treated or untreated cells. The methylation status of ER? in osteosarcoma cells is affected by estrogen. These findings indicate that epigenetic changes of genomic DNA regulate ER? synthesis. Taken together, our results suggest that SAOS-2 cells can be an interesting model for further investigating ER? synthesis. In addition, the evaluation of ER? methylation in osteosarcoma specimens is in progress.

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