Forskolin Stimulates Bone Sialoprotein (BSP) Gene Expression in Human Prostate Cancer Cells

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Purpose: Forskolin (FSK) is cell-permeable diterpenoid that possesses anti-hypertensive, positive inotropic and adenylate cyclase activating properties. The activation of adenylate cyclase results in increased intracellular cAMP in most tissue and cells. Bone sialoprotein (BSP) is a mineralized tissue-specific protein that is glycosylated, phosphorylated and sulfated. BSP is highly expressed during initial formation of bone, and may function in the initial nucleation of hydroxyapatite crystal in \textit{de novo} bone formation. The expression of BSP is regulated by various hormones and growth factors. In this study we used FSK to look at the effects of cAMP on the expression of BSP using human prostate cancer DU145 cells.

Materials and Methods: To determine the molecular basis of the transcriptional regulation of BSP gene transcription by FSK, we conducted real-time PCR (BSR, Runx2, Osterix, estrogen receptor; ER and androgen receptor 1; AR1), transient transfection analyses with chimerical constructs of the human BSP gene promoter linked to a luciferase reporter gene, and gel mobility shift assays using radiolabeled double stranded oligonucleotides (cAMP response element; CRE and FGF2 response element; FRE).

Results: Treatment of DU145 cells with FSK (1 \textmu M) for 6 and 12 h resulted in increased in the mRNA levels of BSP, Osterix, ER and AR1. However, Runx2 mRNA level did not change after stimulation by FSK. In transient transfection analyses, using various sized human BSP gene promoter luciferase constructs, FSK (1 \textmu M, 6 h) stimulated luciferase activity of the construct (-184HumanBSPLUC), which encompasses nucleotides -184 to +60 transfected into DU145 cells. The effect of FSK was inhibited by Protein kinase A (PKA) inhibitor H89. Gel mobility shift assays with two CRE (CRE1: -79\textendash;72 and CRE2: -673\textendash;666) and FRE ds-oligonucleotides revealed increased binding of nuclear proteins from FSK (1 \textmu M, 6 h) stimulated DU145 cells. The CRE1-protein complexes formation was inhibited by anti-CREB antibody and supershifted by anti-phospho CREB antibody.

Conclusion: These studies indicated that FSK (1 \textmu M) increased BSP gene transcription through PKA dependent pathway and that the FSK effects were mediated through CRE1, CRE2 and FRE elements in the human BSP gene promoter.

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