

論文内容要旨

HBp17/FGFBP-1 Expression is Down-regulated by 1 α ,25(OH) $_2$ D $_3$ through NF- κ B pathway in Oral Squamous Cell Carcinoma Cell Lines.

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1. INTRODUCTION

Heparin-binding protein 17 (HBp17) was originally purified from the medium conditioned by human epidermoid carcinoma cell line A431, and later renamed as fibroblast growth factor-binding protein-1 (FGFBP-1). HBp17/FGFBP-1 was initially found to bind FGF-1 and FGF-2 in a non-covalent and reversible manner. Recently, it was reported that up-regulation of HBp17/FGFBP-1 was found in various tumors, including head and neck, skin and colon cancers. Binding of HBp17/FGFBP-1 to FGF-2 mediates the release of immobilized FGF-2 from the extracellular matrix allowing it to bind its receptor, suggesting that HBp17/FGFBP-1 serves as an extracellular pivotal switching molecule for FGF-2.

FGF-2 is the prototypic member of a family containing at least 23 structurally-related polypeptide growth factors. It is expressed ubiquitously in a variety of tumor cells including oral squamous cell carcinomas (OSCC). *In vitro*, FGF-2 is a potent mitogen for different cell types, including vascular endothelial cells, fibroblasts and cancer cells such as OSCC cells. *In vivo*, FGF-2 is a potent inducer of angiogenesis and has pleiotropic effects both on development, differentiation in various organs, and tumorigenesis.

$1\alpha,25$ -dihydroxyvitamin D₃ (VD₃) is a multifunctional hormone that exerts its action through the vitamin D receptor (VDR). The anti-cancer potential of vitamin D analogs on its ability to inhibit growth of cancer cells, induce apoptosis and even inhibit angiogenesis has been reported. One of the mechanisms of VD₃ is by inhibiting NF- κ B activation and signaling. Based on the promoter sequence in the HBp17/FGFBP-1, it has been speculated that the sequence has a possible NF- κ B binding site. Thus, we tested the hypothesis that HBp17/FGFBP-1 expression was regulated by NF- κ B by manipulating this particular binding site, and then studied the possibility to inhibit the activation of FGF-2 in OSCC cells by repressing the expression of HBp17/FGFBP-1 using VD₃.

2. MATERIALS AND METHODS

Oral cancer cell lines; UE: HO-1-u-1 and NA (oral squamous carcinoma cell lines), A431 (dermoid carcinoma cell lines) have been used in this study. The cells were treated periodically with VD3 (40nM) in serum-free defined medium designated RD6F. The mRNA and protein expression for HBp17/FGFBP-1, FGF-2, Vitamin D3 receptor (VDR) and NF- κ B molecules (I κ B α , p65 and p50) were quantified using quantitative real-time PCR (qRT-PCR) and western blotting (WB). Immunofluorescence analysis was performed to reveal the effect of VD3 on the distribution of HBp17/FGFBP-1 in UE cells. Meanwhile, a luciferase reporter assay was performed to determine HBp17/FGFBP-1 promoter activity (region between -217 and +61; region containing deduced NF κ B binding site) in response to the treatment.

Additionally, effect of VD3 on the expression of HBp17/FGFBP-1 and FGF-2 was examined using quantitative enzyme-linked immunosorbent assay (ELISA). VDR RNAi-transfected UE cells (siVDR) were used to determine the role of VDR on HBp17/FGFBP-1 expression by the VD3.

3. RESULTS

HBp17/FGFBP-1 mRNA and protein level were significantly down-regulated by VD3 (40nM), with highest inhibition observed at 12 hour after the treatment. The expression levels of FGF-2, VDR, p65 and p50 did not change after the treatment. Suppression on HBp17/FGFBP-1 expression was also confirmed by immunofluorescence analysis. As a result of a luciferase reporter assay, promoter activity of HBp17/FGFBP-1 was significantly suppressed by the VD3 treatment. On another note, the level of I κ B α , which is known as an NF κ B regulator was up-regulated.

In siVDR cells, HBp17/FGFBP-1 expression was modestly suppressed (20%) by VD3 treatment compared to that of wild type where HBp17/FGFBP-1 expression was inhibited in higher degree (70%). By contrast, I κ B α expression was unaffected in siVDR cells, while its expression was strongly induced (70%) in wild-type cells.

Although VD3 did not show to have direct effect on FGF-2 expression, it has been revealed by ELISA that the level in the medium conditioned by the cells treated with VD3 significantly decreased.

4. DISCUSSION

The data from quantitative RT-PCR, western blotting, and ELISA clearly showed that down-regulation of HBp17/FGFBP-1 resulted in inhibition of the FGF-2 release from the extracellular matrix.

Apart from HBp17/FGFBP-1 down-regulation, analysis of VD3-treated cells revealed an

up-regulation of I κ B α which function as the regulator of NF κ B activity. Such an observation in previous report has been an indicator for an inhibition of NF- κ B activity. The result by the Luciferase reporter assay demonstrated that the speculated NF- κ B binding site in region between -217 and +61 of HBp17/FGFBP-1 promoter sequence is a functional NF- κ B binding site.

In siVDR cells, inhibitory effect of VD3 on the expression of HBp17/FGFBP-1 was diminished compared to that on siControl cells, suggesting these VD3 inhibition on HBp17/FGFBP-1 is mediated by VDR. These results strongly suggest the possibility that 1 α ,25(OH) $_2$ D $_3$ might be a useful agent for the oral cancer therapy by targeting NF- κ B and HBp17/FGFBP-1 molecules.