Transfection of Naked NF kappa B Decoy Oligodeoxynucleotide Suppresses Pulmonary Metastasis by Murine Osteosarcoma in the Alginate-encapsulated Tumor Spheroid Model

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Introduction: Osteosarcoma is the most common primary malignant tumor of bone. Despite multidisciplinary treatment of the tumor, a significant proportion of patients develops pulmonary metastasis and eventually succumbs to the disease. There is a pressing need to develop new approaches to suppress the progression of pulmonary metastasis. The transcription factor, nuclear factor-kappa B (NFkB), is a heterodimeric DNA-binding protein that plays an essential role in tumor progression and metastasis [1]. Blocking the NFkB signaling pathway has been reported to inhibit the metastasis of breast cancer [2] and the invasiveness and motility of osteosarcoma cells [3]. Recently, synthetic double stranded oligodeoxynucleotides (ODN), which act as ‘decoy’ cis-elements to block binding of NFkB to promoter regions of targeted genes, have been developed; the efficacy of ODN in blocking the NFkB target genes has also been demonstrated [4]. We hypothesized that transfecting an osteosarcoma with NFkB decoy ODN (decoy) would suppress its ability to form pulmonary metastases. Using a murine osteosarcoma cell line with a high metastatic potential to the lung (LM8), we have recently established a new alginate-encapsulated tumor spheroid model. The purpose of this study was to examine the effect of NFkB decoy ODN on tumor progression in vitro and pulmonary metastasis in vivo by LM8 cells in the alginate-encapsulated tumor spheroid model.

Materials and Methods: Cell Preparation: LM8 was established from the original Dunn cell line by in vivo selection [5]. LM8 cells cultured on monolayer were digested by 0.05% trypsin and suspended in 1.2% alginate beads at 4.0 x 10^6 cells/ml [6] and cultured in DMEM supplemented with 10% fetal bovine serum (FBS). Transfection of NFkB decoy ODN: Phosphorothioate double-stranded ODN, from which sequences have been reported [4], was used. The scrambled decoy ODN (SCD) was used as a ODN control. After 24 hours of pre-culture in serum-free medium (SFM), ‘naked’ NFkB decoy ODN or SCD were transfected into LM8 cells encapsulated in alginate beads for 4 hours. Transfection efficiency: FITC-labeled decoy ODN was used to determine the transfection efficiency. The fluorescent intensity was observed using confocal microscopy. Nuclei were counterstained with propidium iodide. Experimental groups: The cells in alginate beads were transfected with naked ODN as follows: 1. Control (SFM); 2. SCD (100 nM); 3. Decoy (100 nM); 4. Decoy (10 μM) for four hours. Then beads of all experimental groups were cultured in DMEM with 10% FBS for 24 hours. Cell proliferation assay: On day-2 and day-7, cell proliferation was determined using a colorimetric immunoassay based on the measurement of bromodeoxyuridine (BrdU) incorporation. Quantitative real-time PCR: One day after the transfection of decoy ODN, RNA was extracted and cDNA was synthesized. Quantitative analysis of VEGF and ICAM-1 were performed by real-time PCR. The mRNA levels were normalized by the GAPDH level of each sample.

Results: Cell proliferation: On day-2 of culture, no significant differences in BrdU incorporation were observed among the experimental groups. However, on day-7, cell proliferative activity was mildly suppressed by the transfection of decoy ODN at 10 μM (% increase of day-2; Control: +12.9 ± 3.1%, SCD: +16.0 ± 3.1%, Decoy [100 nM]: +21.5 ± 3.1%, Decoy [10 μM]: +6.3 ± 5.3%, p<0.01 vs. SCD, Decoy [100 nM]).

mRNA expression of VEGF and ICAM-1: The transfection of decoy (10 μM) induced a significant reduction of mRNA levels of both VEGF and ICAM-1 genes, compared with the other groups (VEGF: SCD: +12.7 ± 2.1%; Decoy [100 nM]: +0.7 ± 2.7%; Decoy [10 μM]: -31.2 ± 3.1%, p<0.01 vs. control / ICAM-1: SCD: -0.3 ± 9.1%; Decoy [100 nM]: -5.9 ± 10.8%; Decoy [10 μM]: -34.3 ± 2.6%, p<0.01 vs. control).

In vivo study: There were no significant differences in tumor volume, as well as body weight, among all groups throughout the experimental period. Microscopic analysis of the lungs showed that metastatic lesions were found in all experimental groups. There was, however, a marked reduction in metastases produced by LM8 cells transfected with decoy ODN at 10 μM (Number of nodules: Control: 49.2 ± 10.0, ; SCD: 66.1 ± 16.4, ; Decoy [100 nM]: 40.6 ± 9.2, Decoy [10 μM]: 19.1 ± 7.3, *p<0.05 vs. control, **p<0.01 vs. SCD) (Fig.2).

Discussion: We have successfully transfected ‘naked’ NFkB decoy ODN into LM8 cells cultured in an alginate-encapsulated tumor spheroid model in vitro. Furthermore, the transfection of ‘naked’ NFkB decoy ODN effectively suppressed pulmonary metastasis in the alginate bead transplantation in vivo model. The results for cell proliferation and mRNA expression of VEGF and ICAM-1 in vitro provide biological evidence for the inhibitory mechanism of pulmonary metastasis produced by LM8 cells.


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