

## **EFFECT OF TLR9 LIGANDS IN K7M2 OSTEOSARCOMA CELL LINE AS IN VITRO BONE MODEL**

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### **Introduction**

Bone metastases is one of the most common and painful consequences of tumor progression. The role of a potential microbial infection in bone metastasis formation or malignant disease in general has not been understood yet.

TLR9 agonists induce Th1 type, pro-inflammatory cytokine and chemokine production of immune cells. Since growing evidence suggests that many types of non-immune cells may also express TLR9, we hypothesized that osteosarcoma cells used as an in vitro bone model also express TLR9, and stimuli induced by CpG oligonucleotides can induce their phenotypic and functional alterations.

### **Methods**

K7M2 osteosarcoma cell line were cultured in DMEM based culture media and proliferation of K7M2 cell lines was studied following stimulation with the TLR9 agonists CpG-ODN 1826 and CpG-ODN H154, and CpG-ODN 1612 as control. We used MTT assay for assessing cell viability. The effect of these interventions on the cell morphology was also evaluated by microscopy.

### **Results**

ODN 1826 significantly inhibited the mitochondrial activity of K7M2 osteosarcoma cells ( $p < 0.0001$ , unpaired, two-tailed t test) and induced the phenotypical alteration of K7M2 compared with negative control ODN 1612. ODN H154 also decreased the metabolic activity of K7M2 ( $p < 0.01$ ) and monolayer forming ability. Morphological changes of the activated cell cultures suggested decreased cell adherence to the solid surface.

### **Conclusions**

Taken together, the TLR9 agonists, ODN 1826 and H154, inhibited cell proliferation and altered cell morphology of K7M2 cells. Based on these results we assume that potential bacterial infection during malignances may affect the cells' adherence, potentially leading to phenotypical alteration and metastasis formation in bones.

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