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Oncolytic effect of *VP3* gene of chicken infectious anemia virus on canine mammary tumor cells

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Mammary tumor is the most common malignant neoplasm in female dogs and it is second major cause of death after accident. Current cancer treatment strategies have resulted in an increase in patient survival but most cancer patients relapse and are resistant to subsequent treatments (Chada and Ramesh 2007). Modern cancer drugs mainly target *p53* gene and *bcl2* proteins to cure the tumors.Simultaneously they have side effects because of alteration in these genes. Therefore, there is an urgent need for new treatment strategies and testing of novel therapeutic agents. Gene therapy is a promising strategy to treat malignancy. Studies reveled that *VP3* gene is having oncolytic property and kills tumor cells selectively without affecting normal cells. *VP3* gene works independently from *P53* gene and *Bcl2* protein (Jin *et al.* 2011).This makes *VP3* gene as an alternative option for cancer therapy. The present study includes to know the oncolytic effect of *VP3* gene on canine mammary tumor cells.

Female dogs (25; 7 Mongrel, 12 German shepherd, and 5 Doberman, 1Golden retriever) having mammary tumor were used in this study. All the animals were subjected to surgical intervention for the removal of tumor growth under atropine @ 0.04 mg/body wt i.m., diazepam @ 0.5 mg/body wt i.v. premedication and thiopental sodium i.v. to effect. For the confirmation of malignancy, tumor samples were collected in 10% buffer formalin beside a small piece of tumor collected in a vial containing DMEM supplemented with 2%FBS and 5X antibiotics, viz. streptomycin, penicillin, gentamycin and nystatin for *in vitro* culture of canine mammary tumor cells. Sections of 6 to 8 microns were stained with H&E stain. Microscopically, degree of differentiation, type of growth pattern of malignant cells, degree of malignancy and cellular characteristics were recorded. Canine mammary tumor cells (CMTs) were cultured *in vitro* as per Lee *et al.* (2003). CMT cells in suspension were sub cultured for six passages continuously. After sixth passage CMT in suspension were transfected by *VP3* gene of CIAV, cloned in PcDNA 3.1 eukaryotic expression vector. Lipofectamine (invitrogen) mediated gene-delivery system was used for transfection of *VP3* gene. Oncolytic potential of recombinant PcDNA.ciav. *VP3* was established in CMT by DNA fragmentation using ladder pattern/agarose gel electrophoresis assay and TUNEL assay.

The biopsy specimens for cell culture and microscopic studies were collected aseptically after surgery. The histopathological studies of mammary lesions revealed adenocarcinoma (45%), fibrosarcoma (20%), fibroadenoma (30%) and fibroma (5%). There are several reports regarding the incidence of different types of tumors in dogs and this may be attributed to the fact that breed prevalence varies widely depending on available population in different geographic zones and period (Khimta *et al.* 2010). Cultured cells of biopsy material were passaged serially and survived for 6 months. Cells were grown in suspension culture and one lineage of CMT cells was used to study the oncolytic effect of the virus. Results revealed that initially virus utilizes mitochondria mediated pathway to kill the cells followed by death receptors and endoplasmic reticulum mediated mechanism at later stages (72 h or later). Results also indicated that the *VP3* protein obtained from Indian isolate of CAV can induce cell death among tumor cells of canine origin *in vitro*. The extent of regression by *VP3* gene in this study was 11.9%. Most of the *VP3* protein in CMT cells was expressed in the nucleus, where it aggregated in the form of a fine granular pattern. The results obtained in this study are in the accordance with the finding of Lee *et al*. (2007) in canine neoplasm.

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SUMMARY

Female dogs (25) of different breeds (7 Mongrel, 12 German shepherd, and 5 Doberman, 1Golden retriever)

having mammary tumor were subjects of this study. All the animals were subjected to surgical intervention for the removal of tumor growth under general anaesthesia. Biopsy samples were collected in DMEM supplemented with 2% FBS and 5x antibiotics for cell culture, while for histopathological studies samples were collected in 10% buffer formalin. The histopathological studies revealed adenocarcinoma (45%), fibrosarcoma (20%), fibroadenoma (30%) and fibroma (5%). The cell culture of malignant tumor was performed using standard protocol. After the sixth passage the oncolytic effect *VP3* gene of chicken anamea virus (CAV) was observed in the cultivated cell line and it was revealed that the *VP3* gene of CAV regressed the growth of tumor cells. It was concluded that *VP3* gene of CAV induces-apoptosis in malignant CMT cells and *VP3* protein has great potential in the development of antitumor treatment. The *VP3* gene of chicken infectious anemia virus (CIAV) showed the oncolytic effect, on malignant canine mammary tumor cell (CMTs) and regression effect of *VP3* was 11.9%.

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