IMMUNOHISTOCHEMICAL DETECTION OF P\textsuperscript{53} IN CANINE MAMMARY TUMORS

P. Veena, R.V. Suresh Kumar, K.B.P. Raghavender, Ch. Srilatha and T.S.C. Rao
Sri Venkateswara Veterinary University
College of Veterinary Science, Tirupati- 517 502, India

Received: 29-06-2013
Accepted: 28-12-2013

ABSTRACT

Mammary gland tumors are the most common neoplasms in female dogs and represent the second most frequently recognized tumors in dogs. Incidence of mammary tumor is as high as 50 per cent of all neoplasms in female dogs as compared to breast cancer in women. The prevalence of Estrogen Receptor (ER) Progesterone Receptor (PR) has been reported to be $\leq$ 50% in mammary tumors in dogs and 60-75% in breast cancer in human beings. Apart from hormonal receptors, proliferative markers like P\textsuperscript{53} and C-erb B2 can be measured by using IHC studies which are of prognostic relevance. In the present study IHC studies were carried out on 24 malignant CMTs consisting of adenocarcinoma (20), solid carcinoma (2), mixed mammary tumor (2) to know the rate of proliferation. P\textsuperscript{53} mutation was evident in 5 (20.83%) of 24 CMTs. P\textsuperscript{53} mutation was evident in 27.2% of the tumors that were declared positive for either or one of the hormone receptors. 20% of adenocarcinomas (4) and 50% of solid carcinoma (1) were found positive for P\textsuperscript{53} immunoreactivity. None of the mixed mammary tumors could express the P\textsuperscript{53} positivity.

Key words: Canine mammary tumors, Immunohistochemical detection, P\textsuperscript{53}, Proliferative marker.
Procedure: Four microns thickness sections mounted on to Poly-L-Lysine coated slides and incubated at 37°C for one day and further incubated at 58°C over night. Sections were deparaffinised and dehydrated and dealcoholised for 1 minute and then rehydrated for 10 minutes under tap water. Rinsed with distilled water for 5 minutes. Sections were kept in citrated buffer (1 mM Citrate pH 6.0). Microwave treatment was given for 10 minutes to retrieve the antigenic sites. Sections were allowed to cool down to room temperature for 20 minutes. Sections were rinsed in distilled water for 5 minutes. Sections were rinsed twice in Tris buffered saline for 5 minutes each time. Sections were kept in peroxidase block for 10-15 minutes for blocking endogenous peroxidase enzyme and were rinsed in Tris buffered saline for five minutes thrice to block non specific reaction with other tissue antigens, sections were kept in power block for 15 minutes. Sections were drained and covered with concerned primary antibody (ER, PR, P53, and C-erb B2) for 1 hour to identify tumor markers by antigen-antibody reaction. Sections were rinsed in Tris buffered saline for 5 minutes thrice to washout unbounded antibodies. To enhance the reaction between primary and secondary antibody, sections were incubated with super enhancer for 30 minutes. Sections were rinsed in Tris buffered saline thrice for 5 minutes. Sections were incubated with super sensitive Poly HRP for 30 minutes to elongate chains and also to label the enzymes. Sections were washed under Tap water for 5 minutes. Sections were counter stained with Haematoxyline. Sections were washed under tap water for 5 minutes to washout the excess stain. Sections were air dried, cleared in xylene and mounted with DPX (Shi et al., 1991 Taylor and Cote, 1994).

Scoring method: The intensity of P53 over expression was assessed using a grading system based on the percent of P53 present nuclei from tumor cells. Tumors were assigned a score of 0-3. 0: less than 1% of nuclei stains, 1: 1-10% of nuclei stains, 2: 11-50% of nuclei stains, 3: more than 50% of nuclei stains.

IHC studies were carried out on 24 malignant CMTs consisting of adenocarcinoma (20), solid carcinoma (2), mixed mammary tumor (2). IHC positive reaction which appears as brown dots and it was expressed as mild to moderate nuclear positivity (Fig.1 and Fig. 2). P⁵³ mutation was evident in 5 (20.83%) of 24 CMTs. P⁵³ mutation was evident in 27.2% of the tumors that were declared positive for either or one of the hormone receptors. 20% of adenocarcinomas (4) and 50% of solid carcinoma (1) were found positive for P⁵³ immunoreactivity. None of the mixed mammary tumors could express the P⁵³ positivity.

The P⁵³ gene seems to be highly conserved with a reported gene homology of 81% between canine and human P⁵³ Genes. It is the most frequently mutated gene in human. Mutations and dysregulation of P⁵³ and gene products have been described in cancers in veterinary practice. The biologic functions of P⁵³ include regulation of DNA replication and repair, control of cell cycle check point, initiation of apoptosis and induction of differentiation. These functions make P⁵³ pivotal to the control and normal function of all cells. Loss of P⁵³ function may result in accelerated tumor proliferation. P⁵³ mediates the cell cycle check point function through GADD45, the growth arrest and DNA damage inducible gene. The check point functions identify and repairs the genetic damage. If unable to complete this repair, initiate the cell death pathways. Mutations in P⁵³ will influence the ability of the cell to detect the DNA damage and arrest through GADD45. P⁵³ mutations in tumors contribute to anti apoptotic phenotype of cancer cells. The loss of cells ability to die as a result of P⁵³ function contributes not only to cancer development and progression, but also to the sensitivity of cancer cells to chemotherapy. Abrogation of P⁵³ activity leads to loss of cell cycle control, increasing the risk of additional genetic aberrations in the affected cells and daughter cells, thus enabling malignant transformation, as well as prognosis of tumor cell towards enhanced malignancy (Muto et al., 2000, Morris and Dobson, 2001, Lee and Kweon, 2002).
REFERENCES


