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TWIST1 ONCOGENE: COMPARATIVE STUDIES, SEQUENCE VARIATIONS AND MRNA EXPRESSION IN CAT MAMMARY GLAND CARCINOMAS

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Feline mammary carcinoma (FMC) is highly aggressive, mainly hormone receptor-negative cancer, proposed as a model for poor prognosis human breast cancer (HBC). Germline mutations in *TWIST1* may predispose to breast cancer and when increased in breast cancer cells it has been shown to promote metastatic ability in *in vivo* animal models. The aims of this study were to partially isolate the *TWIST1* gene in *Felis catus*, perform comparative studies, to screen spontaneous FMC for sequence variations and evaluate its mRNA expression. Primer combinations were selected based on the alignments of homologous DNA sequences. After PCR amplification using cat gDNA, 3 bands were obtained (around 300, 800 and 1000 bp), purified and sequenced. Several bioinformatic tools were used to perform comparative studies. 30 spontaneous FMC were screened for polymorphisms. To evaluate the *TWIST1* expression in 7 FMC, RNA extraction/purification and cDNA synthesis were performed. Primers were designed and hybridization probes selected according to major homology for each transcript. There is a higher similarity between the isolated *TWIST1* gene in *Felis catus* and *Homo sapiens* (86%) than between *Homo sapiens* and *Rattus norvegicus* or *Mus musculus* (75%). The partial amino acid sequence showed no change in these four species. This inferred coding sequence presented high similarity (~96%) between *Homo sapiens* and *Felis*

catus. No sequence variations were identified in all tumours analyzed regarding the predicted coding region. *TWIST1* was downregulated in all carcinomas. We believe that this investigation is the first one to study the *TWIST1* gene in cat regarding all the aspects here reported. The comparative studies evidenced a higher similarity between cat and man than between man and other widely used animal models such as rat or mouse. These results suggest that cat is as an attractive model, at least for *TWIST1* studies, and that may be used instead of the classical animal models. *TWIST1* downregulation in all carcinomas is, however, an unexpected result. Nevertheless numbers are small suggesting future directions for further investigations. In conclusion, although we present here some challenging results, we also give the first insights regarding the *TWIST1* gene in cat that may contribute to establish a feline spontaneous model to study HBC.