

7 SUMMARY

Expression profile of the cyclin-dependent kinase inhibitors p21^{CIP1/WAF1} and p27^{KIP1} in spontaneous canine mammary tumors

The cell cycle and its mechanisms of control play an important role concerning the carcinogenesis of epithelial neoplasia. The different regulators of the cell cycle are responsible for its proper progress to assure the accurate doubling of the DNA. These issues in human research document the importance of the expression profile of the cyclin-dependent kinase inhibitors (CKIs) p21 and p27 concerning the carcinogenesis of human mammary tumors. To date the investigation of these two CKIs has been insufficiently in veterinary research and was mainly focused on the protein expression of the two candidates. The goal of this study was to provide insight in the expression profile of p21 and p27.

Mammary carcinomas (simple, solid carcinomas) were investigated in microarray analysis. The results revealed overexpression of p21 and a limited expression of p27 in canine mammary tumors compared with non-neoplastic mamma of the same individual.

25 canine mammary carcinomas and corresponding non-neoplastic mammary tissue of the same individual were taken *intra operationem*, followed by histological classification of tumor species and their quantitative analysis of transcriptional expression concerning p21 and p27.

p21 showed an 1,38-fold higher expression level in canine mammary tumors as in non-neoplastic mammary tissue of the same dog what means that a reliable predication of gene-regulation is impossible.

The expression level for p27 was 0,77-fold in the carcinomas compared to non-neoplastic mammary tissue of the same dog.

Both cyclin-dependent kinase inhibitors did not show regulation in expression concerning their transcription. Only tendential regulation was obvious. p21 showed the tendency for a higher, p27 for a lower gene-expression in the mammary carcinomas compared to non-neoplastic mammary tissue of the same dog.

The expression-analysis at transcriptional level of the two candidates was followed by the randomized immunohistochemical analysis of their protein expression to provide semiquantitative information about the protein expression of p21 and p27.

Immunohistochemical staining concerning p21 indicated a higher expression level of this protein in canine carcinomas whereas for p27 the staining of p27- protein was limited in canine carcinomas compared to non-neoplastic mammary tissue of the same individual.

Thus the results of immunohistochemical investigation and the protein expression of the two candidates differ from expression at the transcriptional level (mRNA expression) shown by real-time PCR.

The results indicate a possible posttranscriptional regulation of these two CKIs.

If it is possible to approve the semiquantitative investigation of the protein level also in quantitative investigations posttranscriptional pathways are likely contributing in the regulation of p21 and p27 protein expression and should be investigated in further studies properly.