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IMMUNOLABELING OF BASEMENT MEMBRANE COMPONENTS IN ADENOCARCINOMAS OF THE MAMMARY GLAND OF THE DOG AND CAT

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Laminin and type IV collagen are intrinsic components of basement membrane (BM) in normal mammary tissue and may be considered the main markers of this structure (2, 5). Changes occur in their distribution and quantity in the transition from normal tissue to carcinoma (6, 7). Immunohistochemistry has proven to be a reliable method of detecting BM (3), so we decided to use it to investigate the patterns of BM deposition in mammary tubular adenocarcinomas of the dog and cat.

Material and methods

Samples of mammary neoplasm from 32 dogs (20 simple type tubular adenocarcinoma, 12 complex type tubular adenocarcinoma) and 18 cats (all simple type tubular adenocarcinoma) plus controls for both species were fixed in buffered formalin, paraffin embedded and cut to 5 µm thick sections. The sections were dewaxed and exposed to 0.04% pepsin (Merck) in 1% acetic acid for 2 hours at 37°C. Polyclonal antibodies to human laminin and type IV collagen had been induced in the rabbit (supplied by Dr. H. Furthmayr - Yale University). Immunohistologic staining was performed by the avidin-biotin peroxidase complex (ABC) method, using a commercial kit (Dakopatt, Copenhagen, Denmark); the staining was developed by 3-3'-diaminobenzidine. The tumors were classified as tubular adenocarcinomas according to Hampe and Misdorp (4) and were chosen for comparative purposes as this type is the most common in the cat and very frequent in the dog.

Results

In the normal control sections the ducts and lobules were found to be surrounded by a thin continuous BM. The same staining pattern was shown by tumor-associated benign epithelial lesions (cysts, ductal hyperplasia, lobular hyperplasia) at the periphery of the tumors. Tubular adenocarcinomas both in the dog and cat did not stain uniformly. Focal regions of well-differentiated carcinoma generally showed the formation of a continuous or partial basement membrane, sometimes thicker than the membrane of normal structures at the periphery of the tumor. Sometimes, however, neoplastic tubules did not stain. Where the neoplastic growth showed an expanded, more solid and poorly-differentiated pattern BM were displaced peripherally or fragmented. Fragments were sometimes thicker than normal membranes. A few tumors had also some papillary aspects with no expression of a BM. No or very little difference was noted in the staining of type IV collagen and laminin.

Discussion

The distribution and quantity of the epithelial BM in malignant neoplasm of dogs and cats varies from normal and shows variations

in different regions of the same tumor. As in the human (2, 7) there seems to be loss of BM components (type IV collagen and laminin) associated with poor differentiation. Barsky et al. (2), however, consider this loss neither a sine qua non nor a pathognomonic sign of malignancy. It probably reflects the loss of tumor ability to synthesize and secrete BM or decreased assembly of secreted components or the acquisition of enzymatic degradation activity (2, 5). Enzymatic degradation could explain the lack of BM staining in some neoplastic structures, even if well-differentiated. The above mentioned hypotheses could also account for the finding of irregular fragments of BM in poorly-differentiated cell clusters. The tumor cells might maintain the ability to produce BM, but might lose their orientation. Fragmentation and absence of a basement membrane barrier may facilitate tumor spread (1).

The thickening of BM in some well-differentiated tumors may be due to hypertrophy (6) of the original structure or further deposition of BM components by tumor cells. Carcinoma cells seem to be able to synthesize laminin *in vivo* (1) and hypertrophy suggests that the normal habit of BM production at the epithelial-stromal interface is often exaggerated rather than lost by malignant epithelial cells (6). The hypertrophied membrane seems to maintain its barrier function and tumor growth and invasion must take place by extension of cell aggregates rather than by single-cell aggregates rather than by single-cell infiltration (6).

As in human cases (7), both in the dog and in the cat, carcinomas with papillary proliferations lacked BM deposition.

Conclusions

On the basis of our results the modifications in BM deposition patterns in mammary adenocarcinomas, in the dog and cat, were the same as those found by different authors (2, 5, 6, 7) in human pathology. As with human neoplasms, also dog and cat tumors show a relationship between malignancy and BM deposition, even though at present the prognostic significance of this observation remains obscure and needs further investigation, especially concerning the potential invasiveness of malignant cells.

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