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Desmoplastic reactions associated with malignant canine mammary tumours#

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Abstract

Desmoplastic reactions or desmoplasia, characterised by proliferation of fibrotic connective tissue around tumour cells, have been described as a prognostic indicator of malignancy in human breast cancers. Desmoplasia ultimately results in deposition of collagen that shows significant differences in its density and orientation at different stages of tumourigenesis. The different collagen patterns associated with tumours are referred to as tumour associated collagen signatures (TACS) and are identified as hallmarks in human tumourigenesis. The present study was aimed to demonstrate the presence of TACS in canine mammary tumours (CMTs). The desmoplastic reactions and collagen deposition occurring in association with CMTs were analysed using routine histopathological, special staining and scanning electron microscopy techniques. Thirty excisional biopsy samples collected from malignant CMTs were utilised for studying the progression of desmoplastic reactions occurring in the tumour associated stroma.

Key words: *Canine mammary tumours, Desmoplasia, scanning electron microscopy, tumour associated collagen signatures*

Collagen present in the cancer associated stroma, which was earlier believed to have a mere passive scaffold role, has recently been recognised as a source of several chemical, biological and mechanical stimuli which modulate tumour invasion and metastasis. Increase in mammographic density is one of the earliest appreciable risk factors associated with the development of human

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breast cancer (HBC). This increased density is largely due to desmoplasia resulting in fibroblast activation and enhanced collagen deposition. Collagen also undergoes remodelling and reorientation during tumourigenesis and hence, deciphering the "collagen code" in cancer progression, could serve as a potential biomarker for prognosis prediction. The different collagen signatures associated with human mammary tumours were designated as tumour associated collagen signatures (TACS) by Provenzano *et al*. (2006) and they categorized the collagen in cancerous glands into three. The increased collagen density adjacent to the tumour was designated as TACS-1, while the straightened or taut collagen stretched around the tumour boundary with fibres running parallel to the tumour border was named as TACS-2. When collagen fibres were seen arranged perpendicular to tumour boundary, it was named as TACS-3. Though the desmoplastic reactions and collagen signatures have been studied to a certain extent in HBCs, the area remains under explored as far as canine mammary tumours (CMTs) are concerned. Hence, the present study was aimed to characterise the desmoplastic reactions histomorphologically and demonstrate TACS in CMTs.

Materials and methods

Excisional biopsy samples obtained from 30 malignant CMTs formed the study material. Samples collected in 10 per cent neutral buffered formalin were subjected to routine histopathological processing and paraffin sections were cut at 4-5 micron thickness and stained using routine haematoxylin and eosin (H and E) staining procedure (Suvarna *et al*., 2018). Histopathological grading of these tumours was done according to Clemente *et al.* (2010). Special stains like Masson's trichrome and Picrosirius red were also employed for demonstrating collagen. Herovici staining technique was used to study neo collagenisation in the stroma of CMTs. (Herovici, 1963; Fitzgerald *et al*., 1996) Scanning electron microscopy was also employed to demonstrate the increase in desmoplasia in tumour tissue in comparison with normal developed mammary gland. The samples (five millimetres in size and two millimetres thickness) were fixed in 2.5 per cent cold glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 hours. Following overnight fixation at 4°C, the samples were washed three times with wash buffer (0.1 M PBS) for 10 min. each. The samples were then dehydrated through serial dilutions of isopropyl alcohol (50 per cent to 100 per cent) each for 45 min. Mounted on the stub with double sided carbon adhesive tape. Samples were then sputter-coated for 3 min with a thin layer of gold (10nm thick layer) for making it conductive using an automated sputter (QUORUM Technologies SC7620, UK). Ultimately, the samples were analysed using a scanning electron microscope. (TESCAN VEGA-3L170) with an accelerating voltage of 10 KV and micrographs were taken at different magnifications.

Results and discussion

In malignant tumours of mammary gland, progression of changes was evident in cancer associated stroma. Majority of the tumours showed increased deposition of collagen and the dense wavy collagen bundles adjacent to the tumour lesion that could serve as reliable hallmark for identifying tumours in early stages, designated as TACS-1, was appreciated (Fig. 1). In large sized tumours which had progressed to higher grades, tensed and straightened collagen was seen aligned parallel to the tumour border and surface epithelium and this alignment constituted the TACS-2 (Fig. 2). The TACS-2 collagen morphology could be due to the stretching of stroma in par with growth of tumour and this might induce a compressive restraint and tensile stress in these expanded fibrils which could in turn stimulate and activate the fibroblasts (Provenzano *et al*., 2006). In certain areas of grade III malignant tumours, re-orientation of collagen was noticed and the collagen fibres were seen arranged perpendicular to the tumour border or surface epithelium. This formed the TACS-3 (Fig. 3), which has been regarded characteristic for high grade invasive mammary gland tumours. Moreover, in some of the most aggressive cases, along with progression to TACS-3, there was a clear loss of appreciable tumour stromal boundary. The stroma was seen infiltrating into the epithelial portion in a manner that could facilitate the collective and easy migration

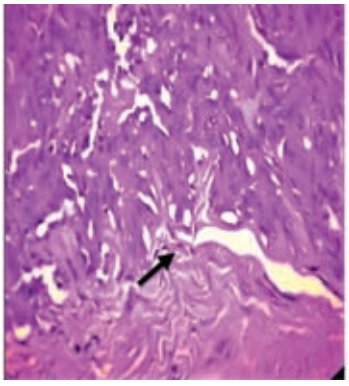

Fig. 1. Wavy bundles of collagen forming TACS-1
(H&E x 100)

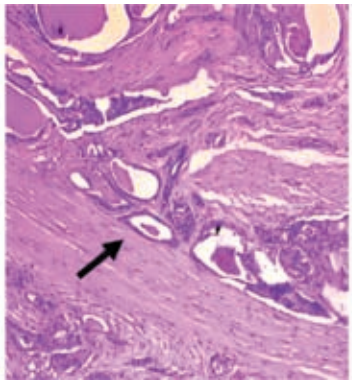

Fig. 2. Linearised collagen arranged parallel to tumour border-TACS-2
(H&E x 100)

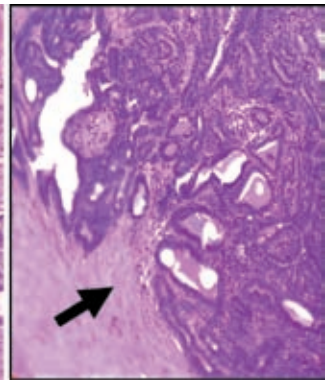

Fig. 3. Collagen arranged perpendicular to tumour border - TACS – 3
(H&E x 100)

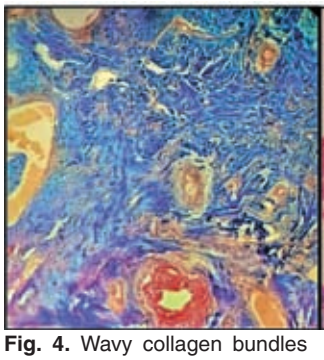

Fig. 4. Wavy collagen bundles -TACS- 1 Masson's trichrome x100

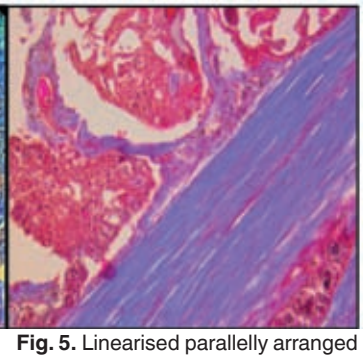

Fig. 5. Linearised parallelly arranged collagen bundles -TACS-2 Masson's trichrome x 100

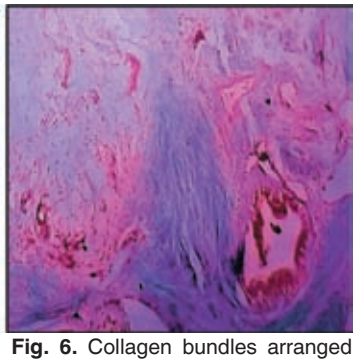

Fig. 6. Collagen bundles arranged perpendicular to the tumour border - TACS- 3 Masson's trichrome x 100

of epithelial cells. The invasiveness of these tumours was evident in thoracic radiographs of the attempted cases. These observations were similar to that of Conklin *et al.* (2011), in HBCs, who reported that TACS-3 was an independent prognostic indicator and Case *et al*. (2017) who identified that grade III HBC cases were less likely to have a tumour-stromal boundary and the lack of a boundary predicted a poor outcome. Apart from mammary tumours, increased desmoplasia and prognostic significance of stromal collagen has been reported in various superficial tumours also (Zainab *et al*., 2019; Shabeeba *et al*., 2021).

Increased desmoplasia, rearrangement and reorientation of collagen during the progression from TACS-1 to 3 was demonstrated using Masson's trichrome straining (Fig. 4 to 6). Picrosirius method of staining was also used to demonstrate collagen arrangement (Fig. 7). With Herovici technique, neo collagenisation was demonstrated and an admixture of blue and green colour with a predominance of blue colour indicative of immature and young collagen was obtained (Fig.8).

Normal mammary gland was compared with cancerous glands using SEM and the sequential desmoplastic changes occurring in the CMT progression were assessed. Normal mammary gland showed less dense and loosely arranged collagen bundles (Fig.9), while the cancerous mammary gland showed thicker and highly dense collagen bundles. An increased deposition of wavy collagen which could be considered as the initial change in early stage tumours, which was clearly appreciated in SEM micrographs (Fig.10). Progression of changes starting from an initial straightening to significant stiffening and linearisation of collagen, which could be

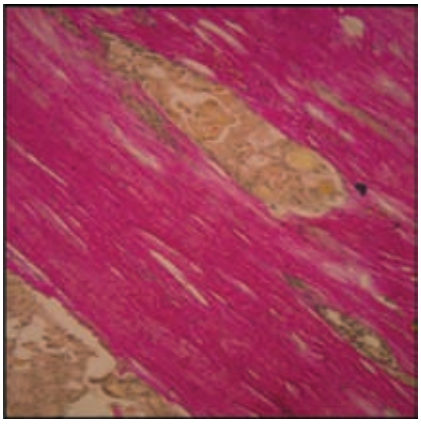

Fig. 7. Linearised collagen Picrosirius red x 100

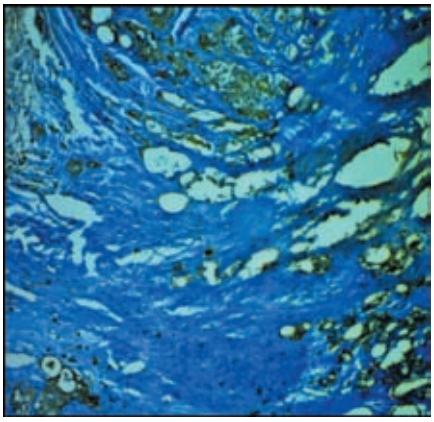

Fig. 8. New young collagen represented by blue colour and neoplastic epithelial cells in yellow colour. Herovici x 100

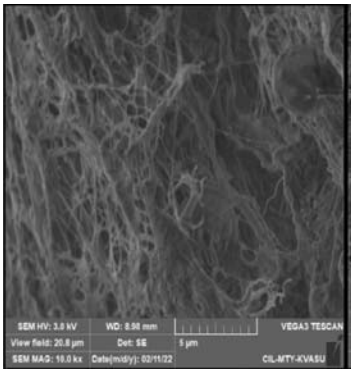

Fig. 9. Normal gland- Loose and less dense collagen SEM 10.0kx

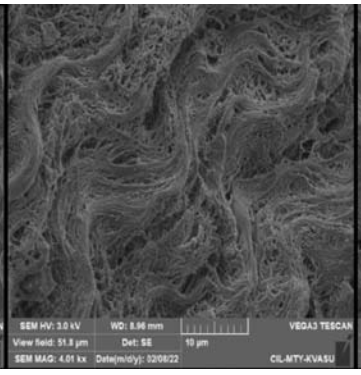

Fig.10. Increased deposition of wavy collagen bundles SEM 4.10kx

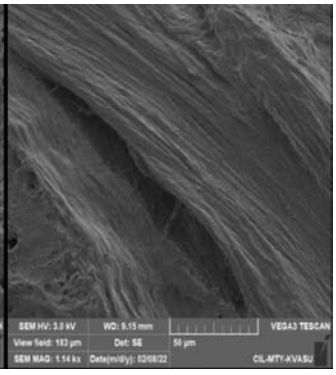

Fig.11. Stiff linearised and fully straightened collagen SEM 1.14kx

SEM 10.0kx

regarded as a feature in advanced tumours, was also visualised (Fig.11). The tumours with linearised collagen corresponded to high grade tumours with clinical evidence of metastasis.

Conclusion

The study identified that altered and increased collagen deposition was central in the progression and metastases of CMTs, which was similar to the observations of Taufalele *et al.* (2019) in HBCs. The three different tumour associated collagen signatures namely TACS-1, 2 and 3, identified as hallmarks in HBCs were appreciated in CMTs also. Hence, TACS analysis may be considered as a potential diagnostic and prognostic tool in navigating the surgeons in decision-making for surgical management of canine mammary malignancies.

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References

Case, A., Brisson, B.K., Durham, A. C., Rosen, S., Monslow, J. and Buza, E. 2017. Identification of prognostic collagen signatures and potential therapeutic stromal targets in canine mammary gland carcinoma. *PLoS ONE* 12(7): e0180448. https://doi.org/10.1371/journal.pone.0180448.

Clemente, M., Perez-Alenza, M. D., Illera, J. C. and Pena, L. 2010. Histological, immunohistological, and ultrastructural description of vasculogenic mimicry in canine mammary cancer. *Vet. Path*. **47**: 265-274.

Conklin, M. W., Eickhoff, J. C., Riching, K. M., Pehlke, C. A., Eliceiri, K. W., Provenzano, P. P., Friedl, A., & Keely, P. J. 2011. Aligned collagen is a prognostic signature for survival in human breast carcinoma. *Am. J. Pathol.* **178**(3): 1221–1232.

Fitzgerald, A.M., Kirkpatrick, J.J., Foo, I.T., Naylor, I.L. 1996. Human skin histology as demonstrated by Herovici's stain: a guide for the improvement of dermal substitutes for use with cultured keratinocytes? Burns. **22**(3):200-202.

Herovici, C. 1963. A polychrome stain for differentiating precollagen from collagen. Stain Technol. **38**: 204–206.

Provenzano, P. P., Eliceiri, K. W., Campbell, J. M., Inman, D. R., White, J. G. and Keely, P. J. 2006. Collagen reorganization at the tumour-stromal interface facilitates local invasion. *BMC Med*. **4**(1):38. doi: 10.1186/1741-7015-4-38. PMID: 17190588; PMCID: PMC1781458.

Shabeeba, P.M., Sajitha, I.S., Devi, S.S., Divya, C. and Nair, S.S. 2021. Histopathological study of canine hepatoid gland tumours. *J. Vet. Anim. Sci.* **52**(3): 277-280.

Suvarna, K., Layton, C. and Bancroft, J. D. 2018. *Bancroft's Theory and Practice of Histological Techniques*. (8th Ed.). Elsevier Health Sciences, 672p.

Taufalele, P. V., VanderBurgh, J. A., Muñoz, A., Zanotelli, M. R., and Reinhart-King, C. A. 2019. Fiber alignment drives changes in architectural and mechanical features in collagen matrices. *PLoS One* 14:e0216537. doi: 10.1371/journal.pone.0216537.

Zainab, H., Sultana, A. and Shaimaa. 2019. Stromal desmoplasia as a possible prognostic indicator in different grades of oral squamous cell carcinoma. *J. Oral Maxillofac. Pathol*. **23**(3): 338-343

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