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Original articles

The skeletal framework of human kidney and renal cell carcinoma

A scanning electron microscopic study

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Summary. The three dimensional architecture of the connective tissue framework of normal human kidney and three renal cell carcinomas was studied. A sodium hydroxide maceration technique was used to remove the cellular elements thus exposing the underlying connective tissue structures. The collagen fibrillar network was visualized using the scanning electron micriscope. In normal kidney the fibres were fine, and smooth, and corresponded to the shapes of the original parenchymal constituents. The fibres of the kidney tumours were coarse in nature and irregularly distributed. The technique provides a rapid method for studying connective tissue fibres in normal and diseased tissue. The three dimensional architecture thus exposed enhances our knowledge of tumour stroma.

Key words: Kidney – Tumour – Connective tissue – Sodium hydroxide – Scanning electron microscopy

The fibroblastic stroma of tumours originates in the connective tissue of the host and has attracted attention for many years. It is moderate in amount in most tumours but very abundant in scirrhous carcinomas. In highly anaplastic carcinomas it is scanty and ill-defined; the stromal components of the invaded tissue may largely disappear. In scirrhous tumours, fibroblastic proliferation produces fibrous tissue in excess of that present in the original tissues both in quantity and quality.

The architecture of the fibroblastic skeleton of tumours varies with the nature of the tumour and the nature of the invaded tissue. Some carcinomas are clearly alveolated or clumped in structure, the epithelial cells being set in spaces of a well-defined stromal network. In anaplastic tumours the sharp line of demarcation between the tmour parenchyma and its stromal skeleton is not present. Astley Cooper, in 1821, pointed out that if you were to macerate scirrhous tumour you might pick out from the cellular tissue the scirrhous substance, and it would have the appearance of a honeycomb. Several attempts have been made to remove cellular elements thus exposing the connective tissue framework using acetic acid, trypsin, EDTA, and low temperature hydrochloric acid [3]. These attempts were not satisfactory. Recently a method has been described using low temperature sodium hydroxide [4]. This has been shown to be effective and consistent in removing cellular elements and thus exposing the underlying connective tissue scaffolding [5]. Using scanning electron microscopy (SEM) the three dimensional arrangement of the connective tissue fibres can be visualized. This is the first time this technique has been used to study the connective tissue framework of normal kidney and kidney tumour.

Materials and methods

The kidney tissue and three renal cell carcinomas were obtained from three patients following radical nephrectomy for renal cell carcinoma. The kidney tissue and tumour were fixed in 10% formalin for a minimum of two days. They were then cut into small pieces measuring 5.5.1–2 mm and fixed overnight in 10% formalin. The cellular elements were then removed by treatment of the tissue pieces with an aqueous solution of 10% sodium hydroxide for three to four days at 25 °C [4]. This solution was changed daily. At the end of the digestion the tissue pieces were washed throughly for several hours, in distilled water, and immersed in a 1.5% aqueous solution of tannic acid overnight. After repeated rinsing with distilled water they were post-fixed in a 1% aqueous solution of osmium tetroxide for three hours. The tissue specimens were then dehydrated in a graded series of acetones and critically point dried (Polaron E3000) using liquid CO_2. The dried specimens were mounted on brass stubs with double sticky tape and coated with gold in a Polaron E5100 sputter coater. They were then scanned in a JOEL 35C scanning electron microscope at 15 KV. Further pieces of tissue were fixed in 2.5% buffered glutaraldehyde and prepared in the standard manner for SEM. Paraffin sections for light microscopy were stained with haematoxylin and eosin and connective tissue stains i.e. van Gieson and reticulin (Gordon and Sweet) stains.

Results

On light microscopic examination of the renal cell carcinomas two types were seen, the clear cell and the papillary

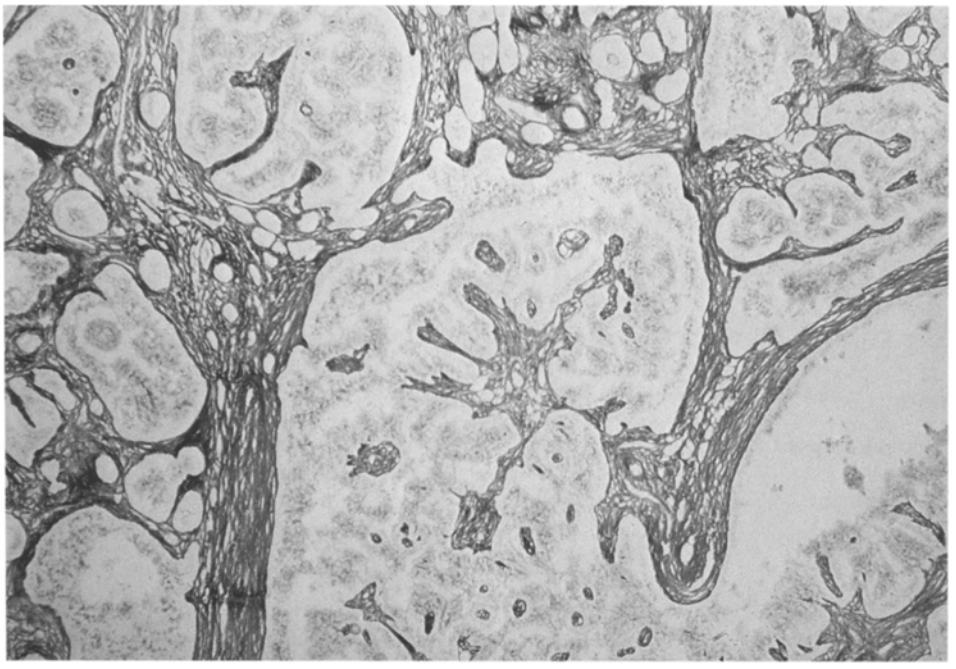

Fig. 1. A light micrograph of a reticulin stained section through the papillary tumour. The papillary stalks which are present in the centre of the picture give the tumour its name. × 165

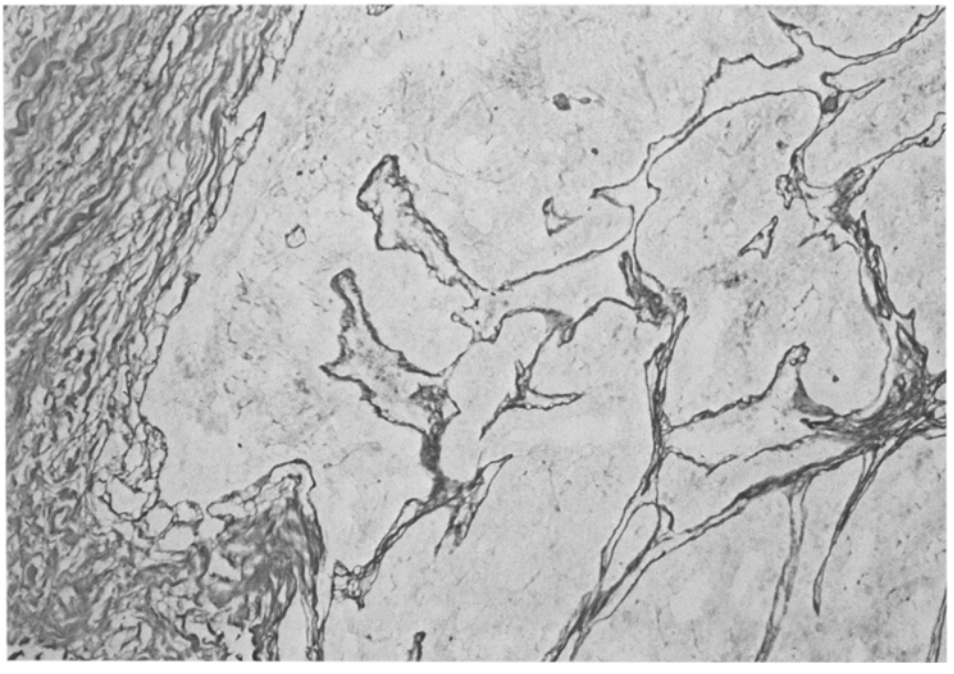

Fig. 2. A light micrograph of a reticulin stained section through the clear cell tumour. × 165

type. Both types were situated at the upper pole of the kidney. The papillary tumour was necrotic on gross inspection while the clear cell carcinoma showed no evidence of necrosis. Connective tissue stains confirmed that the skeletal framework in both tumours was collagenous in nature as shown in Figs. 1 and 2. It is evident from the SEM micrographs that the sodium hydroxidemaceration technique was effective in removing the cellular elements and basal laminae thus exposing the underlying connective tissue framework. With this technique the fibres were not distorted, they were preserved in their original locations and shapes. In previous studies [5, 8] transmission electron microscopy confirmed that the exposed fibres are collagenous in nature and not altered by the sodium hydroxide maceration technique.

Figure 3 is a scanning electron micrograph of normal kidney tissue. This has been prepared in the standard manner for scanning electron microscopy: it has not been treated with sodium hydroxide. It is a section through the renal cortex demonstrating the glomerulus inside Bowmans capsule and cross sections through the proximal tubules. The three dimensional organization of the collagen fibrillar network of normal human kidney is shown in Fig. 4. Following removal of cellular elements using the

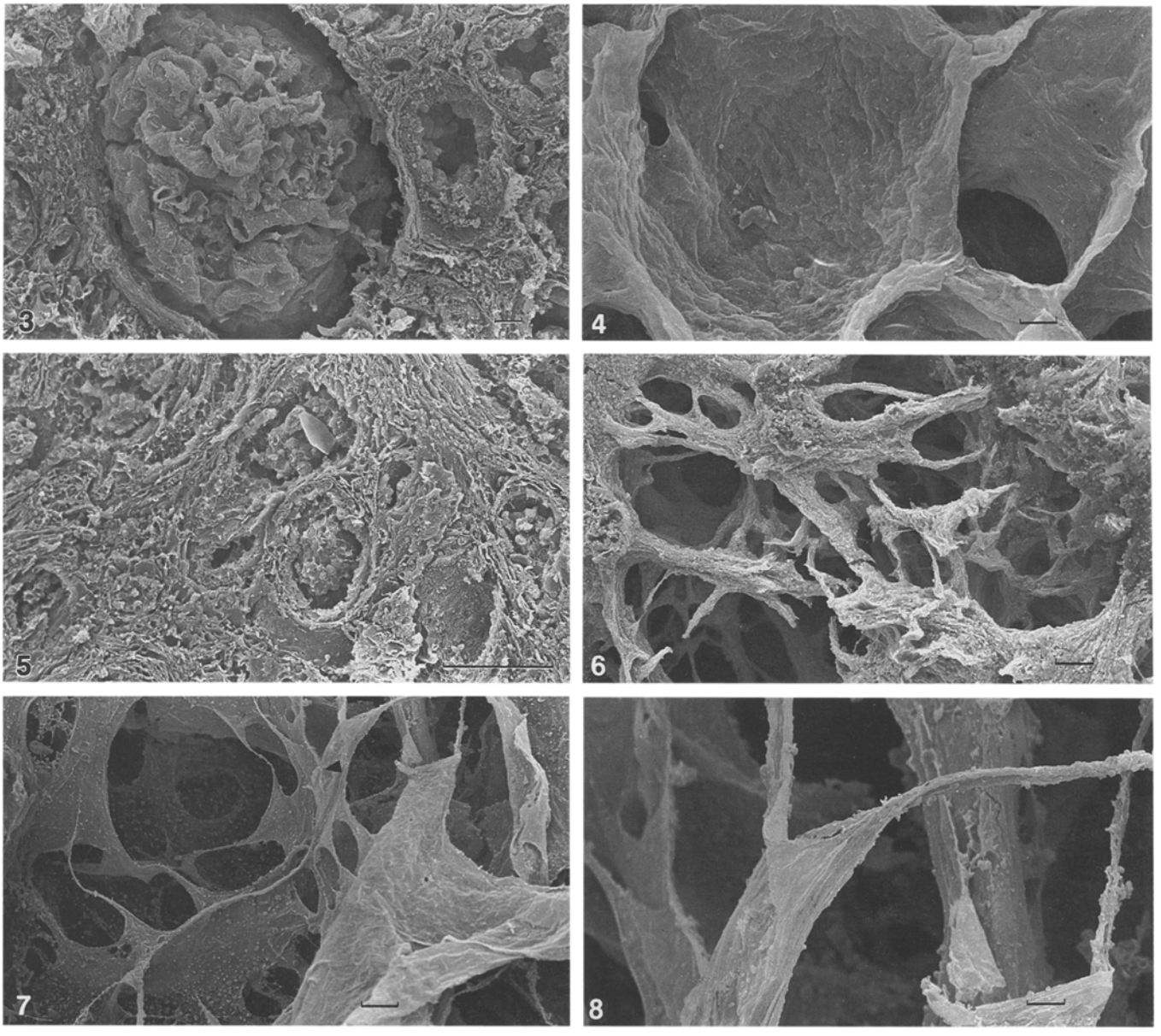

Fig. 3. SEM view of the renal cortex, demonstrating a cross section through a renal glomerulus and tubules. ×658. Bar = 8 μm

Fig. 4. SEM view of the cut surface of the human kidney treated with sodium hydroxide. This reveals the connective tissueframework which surrounds the renal tubules and glomerulus. ×658. Bar = 8 μm

Fig. 5. SEM view of the cut surface of a papillary typerenal cell carcinoma. A small portion of the renal capsule which had been penetrated by tumour is visible in the upper right photograph. ×174. Bar = 32 μm

Fig. 6. SEM view of a papillary type renal cell carcinoma which has been treated with sodium hydroxide. Note the coarse nature of the connective tissue fibres, their irregular distribution and the presence of papillary stalks. ×174. Bar = 32 μm

Fig. 7. SEM view of clear cell type renal cell carcinoma treated with sodium hydroxide. Note the fine nature of the fibres in comparison with those seen in Fig. 5. ×225. Bar = 25 μm

Fig. 8. Higher magnification of area represented by arrow in Fig. 7. This higher magnification emphasizes the fine texture of the fibre. ×114. Bar = 8 μm

sodium hydroxide maceration technique, the highly organized tissue network that normally supports the renal tissue remains. The spaces demarcated by the collagen fibres were occupied by the proximal tubules and glomerulus in the natural state. This network is fine and smooth in texture. The glomerulus is surrounded by a very definite connective tissue capsule, at the posterior aspect of which is the foramen for the afferent and efferent arteriole. The alveolated nature of the two renal cell carcinoma types is obvious in Figs. 5–8. Figure 5 is a section through the papillary type tumour which has not been treated with sodium hydroxide. The superior aspect of this micrograph demonstrates the fibres of the tumour capsule running transversely which have been penetrated by clumps of tumour cells. In the corresponding section (Fig. 6) whichhas been treated with sodium hydroxide the connective tissue skeleton can be examined. In contrast to the fibres in the normal kidney those of the papillary tumour are coarse in nature and irregularly distributed. The normal

architecture has been completely disrupted. The projections from the main framework correspond to the papillary stalks as seen on light microscopy. The collagenous framework of the clear cell tumour (Fig. 7) is morphologically different from the papillary tumour. The fibres although haphazardly arranged bear some resemblance to the type of fibre seen in the normal kidney. The architectural distortion of the fine texture of the fibres is emphasized in the higher magnification view seen in Fig. 8.

In the scanning electron micrographs of normal kidney and tumour, which had not undergone the maceration process, the connective tissue framework was completely obscured by cellular material, as seen in Fig. 3.

Discussion

This is the first time this technique has been applied to the study of normal human kidney or tumour stroma. Ribbert was the first to postulate that connective tissue plays a role in carcinogenesis [6]. This has been alluded to by many authors since then but knowledge on the subject is limited. Pathologists have noted that malignant changes in epithelium were preceded by definite alterations in the underlying stroma. These changes included swelling, disintegration and loss of collagen, activation of fibroblasts, enhanced polysaccharide staining and a rise in mast cell counts [7].

This is the first time that the morphological nature of the collagen fibres in human kidney, kidney tumour and capsule has been demonstrated. The sodium hydroxide maceration technique is the most effective method known for exposing the connective tissue fibres without distortion of their natural shape or location [5]. The complex cellular arrangement of the normal kidney is suspended in and held together by a highly structured network of fibres. In the case of renal cell carcinoma the normal cellular structure is completely disrupted and the nature of the fibre varies from one type of tumour to another and within the same tumour.

The skeletal framework of renal cell carcinomas of both the papillary and clear cell type reflects the clearly alveolated structures of the neoplasm. The collagen fibres of the necrotic papillry tumour are coarse in appearance and totally unlike those of the normal kidney. The fibres of the clear cell tumour although haphazardly arranged are fine, and smooth, and bear some resemblance to the type of fibre seen in the normal kidney. All malignant neoplasms possess a non-neoplastic stroma derived from blood vessels and connective tissue of the invaded structure. This stroma provides the vascular supply that tumours require for nourishment, gas exchange and waste disposal; it may limit the influx of inflammatory cells thus providing a barrier to immunological rejection [1, 2].

Conventional methods of investigation have until now failed to demonstrate adequately the nature of this skeletal framework which is so vital to the growth and invasive properties of the tumour. The application of the light microscope to this type of study is limited as cellular material present obscures detail. For the same reason SEM of normal tissue is similarly limited. The light microscope will tell only of the amount of connective tissue present, it gives no idea of the morphological type of fibre.

This technique provides a rapid method for demonstrating the connective tissue fibres in normal and diseased tissue, it enhances our knowledge of tumour stroma and thus to some extent our understanding of the biology of tumour growth.

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